FACTORS AFFECTING THE
PERIPHERAL 5'-MONODEIODINATION
OF THYROXINE

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1981

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the guidance of my Chairman, Dr. Melvin J. Fregly, who not only allowed me the latitude and support to initiate independent projects, but also encouraged me to view individual experiments as building blocks in the resolution of broad scientific questions. I am indebted to the other members of my committee, Drs. Baker, Cassin, Gerencser and Shiverick, all of whom have generously lent equipment and chemicals to me and provided encouragement and criticism, when either was warranted. I am especially grateful to Drs. Baker and Shiverick, who allowed me the priviledge of working closely with them in their laboratories. In addition, thanks are due to members of the laboratory for their help in conducting this research: Thomas Connor, Charlotte Edelstein, Howard Clark, Christopher Barney, Dianne Kikta and Juan Posada.

Lastly, I wish to thank my wife, Cindy, who unselfishly gave of her time during my graduate training and completion of this dissertation in spite of the demands of her own career.

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Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

FACTORS AFFECTING THE PERIPHERAL 5'-MONODEIODINATION OF THYROXINE

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Extrathyroidal 5'-monodeiodination of thyroxine (T_4) accounts for most of the daily production of triiodothyronine (T_3) in the rat and T_3 is considered to be the more potent of the thyroid hormones. An *in vitro* system, developed to study the enzymatic nature of this process, has demonstrated that a large proportion of peripheral 5'-monodeiodination occurs in liver and kidney. The purpose of these studies was to assess the effects of acclimation to cold, renal hypertension, and chronic administration of either estrogen, aminotriazole, or theophylline on the concentrations of T_3 and T_4 in the serum and on the rate of 5'-monodeiodination of T_4 to T_3 by 2,000 x g supernatants of hepatic and renal homogenates. Cold acclimation was accompanied by an increase both in peripheral 5'-monodeiodinase activity and in the concentration of T_3 in the serum. On the other hand, renal encapsulation and administration of aminotriazole resulted in a reduction in these parameters. However, alterations in the rate of peripheral 5'-monodeiodination of T_4 resulting from administration of either theophylline or estrogen were not consistently accompanied by alterations in the concentration of T_3 in the serum. The increased thyroid activity observed in cold-acclimated rats appeared to be responsible for the increased T_4 5'-deiodinase activity of these animals, while a decrease in thyroid hormone secretion in aminotriazole- and theophylline-treated rats most probably resulted in the decreased rate of conversion of T_4 to T_3 in hepatic and renal homogenates from these animals. The results of these studies have demonstrated the difficulty in correlating the rate of deiodination of T_4 to T_3, as measured *in vitro*, to that which occurs *in vivo* and to the serum concentrations of T_4 and T_3. Factors which possibly contribute in this respect are discussed.

GENERAL INTRODUCTION

An understanding of the mechanisms by which hormones are transported, distributed, metabolized and excreted is necessary in order to comprehend their biological action and therapeutic usefulness. The factors affecting the biological action of thyroid hormones are no exception in this respect, and they have been the subjects of several excellent reviews (1, 2). In recent years, the advent of specific radioimmunoassays for thyroid hormones and their metabolites, and the ability to measure more precisely the enzyme systems responsible, have enabled investigators to assess further the extent of metabolism of thyroid hormones and the factors capable of modulating it. Studies of this kind are potentially of considerable importance, as certain of the metabolites of thyroxine, the major secretion of the thyroid gland, are biologically very active.

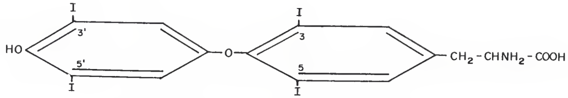
The physiological actions of thyroid hormones are generally diffuse and take several days for an effect to be manifested. Therefore, their role appears to be more in establishing and permitting long term functions and adaptations than moment to moment regulation. In mammals, thyroid homones are required for normal growth (3), for promotion of skeletal maturation (4), and for normal development of the central nervous system (4). In addition, thyroid hormones increase the rate of oxygen consumption (5), stimulate the cardiovascular system, accelerate the catabolism of glucose and fat, and stimulate protein synthesis (6).

Therefore, it is evident that a change in the metabolism of thyroxine to either a more or less biologically active metabolite might have a profound effect on the growth, development and functioning of the organism.

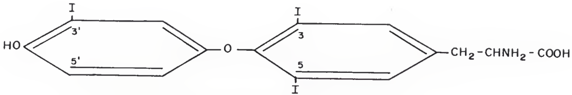
Historical background

Thyroxine was first isolated in crystalline form from the thyroid gland in 1915 (7). In this study Kendall described the hormone as an iodinated oxyindole and proposed the name "thyroxin," derived from the combination of thyroid and oxyindole. Twelve years later Harington and Barger (8) synthesized thyroxine and demonstrated that the structure was actually that of l-3,5,3',5'-tetraiodothyronine (T_4). However, the name thyroxine has been retained. Simultaneously, Lyon (9) reported that synthetic T_4 was as effective in raising the basal metabolic rate of hypothyroid patients as material extracted from thyroid tissue, suggesting that T_4 was the sole active hormone of the thyroid gland. However, a second thyroid hormone, l-3,5,3'-triiodothyronine (T_3), was isolated in 1952 from human plasma and in 1953 from rat and bovine thyroid tissue by Gross and Pitt-Rivers (10, 11). As shown in Figure 1, T_3 differs from T_4 only in the absence of one iodide atom from the 5' position of the outer ring.

Since the discovery of T_3, a number of studies have investigated the source of this hormone. In laboratory animals, the concentration of T_3 is higher in the venous effluent from perfused thyroid glands in situ than in the arterial blood (12, 13). Furthermore, changes in the T_3/T_4 ratio in the thyroidal pool of non-thyroglobulin-bound iodothyronines are reflected by equivalent changes in the ratio of these hormones in the venous effluent of the gland (14, 15). The results of



T_4, THYROXINE (3,5,3',5' - TETRAIODOTHYRONINE)



T_3, (3,5,3'- TRIIODOTHYRONINE)

Figure 1. Stuctural formulae of thyroxine (T_4) and triiodothyronine (T_3).

these studies indicate that at least a portion of the T_3 in the blood results from synthesis and direct secretion by the thyroid gland.

On the other hand a number of studies have focused on the possibility that a fraction of T_3 in the blood of humans and laboratory animals arises from extrathyroidal monodeiodination of T_4. In 1955 Pitt-Rivers _et al_. (16) detected small amounts of T_3 in the blood of athyreotic humans after administration of T_4. However, the claim of these investigators that T_4 was converted to T_3 by the peripheral tissues in man was subsequently retracted (17), and the likelihood of metabolically significant conversion of T_4 to T_3 in man was generally discounted. These studies employed the technique of administering ^{131}I-labeled T_4 in one injection, followed by attempts to identify ^{131}I-labeled T_3 in the serum. Pitfalls in the chromatographic method for analysis of the thyroid hormones were most probably responsible for the conflicting results. However, more recently Braverman _et al_. (18) analyzed the total serum T_3 and T_4 in hypothyroid patients maintained on pharmacological doses of T_4. Using more exact chromatographic methods, these investigators found that the T_3:T_4 ratios in the sera were considerably higher than the ratio in the ingested T_4 preparation. Subsequently, Sterling _et al_. (19) and Pittman _et al_. (20) have reported the conversion to T_3 of isotopically-labeled T_4 administered intravenously to normal human volunteers. These findings have provided evidence of extrathyroidal conversion of T_4 to T_3 in man.

Evidence that T_3 is derived from T_4 by deiodination in extrathyroidal tissues of laboratory animals has also been abundant. In 1951 Gross and LeBlond (21) demonstrated that ^{131}I-labeled T_4 was metabolized in thyroidectomized mice to yield at least two unknown compounds, one of which was shown later to be T_3 (10). Several years later, Flock and Bollman (22) reported that deiodination of T_4 in the rat was reduced by the removal of the liver, and Kalant et al. (23) demonstrated the presence of labeled T_3 in homogenates of liver from rats after administration of ^{131}I-labeled T_4. Furthermore, Becker and Prudden (24) have reported deiodination of T_4 to T_3 by the isolated perfused rabbit liver. The latter three studies were the first to describe the liver as a possible site of conversion of T_4 to T_3. In addition, in 1954 deiodination of T_4 was demonstrated in renal slices of the rat in vitro (25). This finding was confirmed a year later by Larson et al. (26), who also demonstrated that the rate of deiodination of T_4 by the kidneys of rats is dependent on the thyroid state of the animal. It was not until twenty-two years later that this important finding was confirmed. In addition, Schwartz et al. (27) demonstrated the presence of ^{125}I-labeled T_3 in homogenates of the total carcass of rats after administration of ^{125}I-labeled T_4.

Therefore, by 1970 it had been established that T_4 is metabolized to T_3 by peripheral tissues, including the liver and kidney, and that this process contributes at least a portion of the T_3 to the serum pool of this hormone. Since that time several studies have attempted to assess what proportion of the T_3 in serum is secreted by the thyroid gland and what proportion is derived from peripheral metabolism of T_4. Using kinetic analysis of total iodothyronine turnover, Abrams and Larsen (28) demonstrated that two-thirds of the T_3 in the serum of rats arises from peripheral monodeiodination of T_4. A similar calculation for man has indicated that this process contributes between 60 and 80% of the total amount of T_3 utilized per day (29, 30).

In many of the studies cited, it was recognized that the conversion of T_4 to T_3 was of some significance in the action of thyroid hormones, as T_3 had been demonstrated to be biologically more active than T_4. For example, in 1952 T_3 was shown to be three times more effective than T_4 in returning the basal metabolic rate of hypothyroid patients to normal (31). A year later T_3 was found to possess five times the activity of T_4 when assayed by the goiter-prevention test in thiouracil-treated rats (32). Recent evidence, while confirming the earlier studies, has demonstrated that various tissues have different sensitivities to T_3 and T_4 (33). For example, T_4 plays a very minimal role in stimulating the thyroid hormone-sensitive hepatic mitochondrial enzyme, α-glycerophosphate dehydrogenase, but rather requires conversion to T_3 for this effect. On the other hand, the release of thyrotropin from the anterior pituitary gland is inhibited by T_4, albeit at a concentration tenfold higher than that required for T_3.

Indeed, the biological potencies of T_3 and T_4 are so different that it has been suggested that T_4 possesses no activity of its own, and that any effect manifested by T_4 results first from monodeiodination to T_3 by the peripheral tissues and subsequent action by this iodothyronine (34, 35). Thus, T_4 has been described as a "prohormone." However, rats, which require thyroid hormones for survival in a cold environment, remain healthy under these conditions when administered both T_4 and propylthiouracil, a compound which inhibits thyroid hormone synthesis and the conversion of T_4 to T_3 by peripheral tissues (36). In addition, Goldfine _et al_. (37) have demonstrated that T_4 increases the uptake of cycloleucine by isolated rat thymocytes, which do not exhibit T_4 to T_3 converting activity. Thus, these studies suggest that T_4 has intrinsic biological activity (albeit less than T_3) and does not always require conversion to T_3 for its action. Furthermore, specific high affinity binding of T_4 to hepatic and renal nuclear fractions has been demonstrated, even though the apparent equilibrium dissociation constant for T_4 was tenfold higher than that for T_3 (38, 39). Therefore, the question arises as to what is the contribution of T_4 compared to T_3 in the overall biological effect of thyroid hormones at physiological concentrations. Assuming that concentration of free T_4 in the plasma is fivefold higher than that of T_3, that the physiological effect is related to the concentration of iodothyronine bound to the nuclear receptor, and that the iodothyronine in plasma is in equilibrium with nuclear iodothyronine, Samuels and Tsai (39) have calculated that T_4 contributes one third or less of the biological activity of the thyroid hormones.

Studies of the Thyroxine 5'-Monodeiodinase Enzyme

Although the existence and importance of extrathyroidal conversion of T_4 to T_3 had been recognized for many years, it was not until 1975 that Visser et al. (40) and Hesch et al. (41) independently established in hepatic homogenates of the rat that this reaction was enzymatic in nature. These investigators employed a specific radioimmunoassay to detect T_3, when the homogenates were incubated with T_4. This technique has become a well established method for measurement of the rate of conversion of T_4 to T_3. Using this method Chopra (42) studied further the nature of the conversion of T_4 to T_3 and the factors capable of modulating it. It was demonstrated that the converting activity of homogenates of liver from rat is influenced by the concentration of homogenate and substrate (T_4), and by the pH, duration and temperature of incubation. 3,3',5'-Triiodothyronine (reverse T_3) was shown to be a competitive inhibitor and the antithyroid agent, propylthiouracil, to be an uncompetitive inhibitor of the conversion of T_4 to T_3 by hepatic homogenates. In addition, homogenates of liver were found to be at least sixteenfold more active than homogenates of muscle, heart, spleen, lung, brain or intestines, while homogenates of kidney were twice as active as those of liver. More recently, the anterior pituitary gland has been shown to possess a T_4 to T_3 converting activity one third to one half as active as the liver (43). However, while intrapituitary conversion of T_4 to T_3 contributes substantially to the nuclear T_3 in this tissue, the small mass of this tissue compared to that of the liver or kidney would suggest that it does not contribute significantly to the circulating pool of T_3. Rather, it appears that the kidneys and liver are most active in this respect.

Studies to examine the subcellular localization of the T_4 5'-monodeiodinase activity of the kidneys and liver have yielded interesting results. Attempts to localize an enzyme using markers known to associate with certain particulate fractions are dependent on the assumption that the enzyme is an integral protein in the membrane, critical to the structural integrity of membranes and not dissociated from the membrane upon disruption of the cell (44). The finding that the T_4 5'-deiodinase activity is localized in the particulate and not the cytosolic fractions of homogenates of liver and kidney indicates that this assumption is valid in this case (45-48). Thus, Leonard and Rosenberg (45) have demonstrated that 80% of renal T_4 5'-deiodinase activity is associated with ouabain-sensitive (Na^+/K^+)-ATPase, a known plasma membrane enzyme, and the remainder is associated with the microsomal fraction. However, it appears that the subcellular distribution of the hepatic T_4 5'-deiodinase differs from that of the renal enzyme. Visser et al. (46) have demonstrated that the enzyme converting system is located in the microsomal fraction of homogenized liver. This finding has been confirmed by Fekkes et al. (47) and Maciel et al. (48), who found that the subcellular distribution of the 5'-deiodinase correlated best with that of glucose-6-phosphatase, a marker enzyme of the endoplasmic reticulum. In addition, Fekkes et al. demonstrated a significant negative correlation, but Maciel et al. demonstrated a significant positive correlation, between T_4 to T_3 converting activity and 5'-nucleotidase, an enzyme marker of the plasma membranes. Although different methods used by these investigators for preparation of the plasma membranes are most likely responsible for the disparity between these studies, the possibility that the 5'-monodeiodinase enzyme is located in the plasma membranes of the liver remains uncertain. However, it is fairly certain that the majority of the enzyme system is distributed in the plasma membranes of the kidney and in the microsomes of the liver. Despite the different subcellular locations of the enzyme in these two tissues Kaplan et al. (49) have shown that the K_m for T_4 in renal and hepatic homogenates was similar, and that the rate of conversion of T_4 to T_3 by the two tissues was similarly reduced by addition of the antithyroid agent, propylthiouracil, and simiarly stimulated by addition of the sulfhydryl protecting agent, dithiothreitol, to the incubation mixtures. The results of this study suggest that the T_4 5'-monodeiodinases of the rat liver and kidney are the same enzyme.

The observation by the latter investigators that the rate of 5'-monodeiodination of T_4 is stimulated by a thiol protecting agent is one of several studies which has led to a tentative model of this enzyme-catalyzed reaction. In 1976 Visser et al. (46) were the first to demonstrate the importance of thiol groups in this reaction. These investigators showed that the ability of various fractions of hepatic homogenates from rats to catalyze the conversion of T_4 to T_3 was lost on subcellular fractionation. However, activity was restored in part by addition of the cytosol to the microsomal fraction. As the apparent necessity for the presence of the cytosol could be circumvented by the incorporation of dithiothreitol into the medium, it was suggested that sulfhydryl groups play an essential role in the enzymatic conversion of T_4 to T_3 and that the cytosol contains at least one cofactor which is a thiol protecting agent itself. This hypothesis has now been strengthened by the results of several studies. Chopra (50) has shown that other thiol-protective agents, mercaptoethanol and reduced glutathione in addition to dithiothreitol, stimulate the rate of monodeiodination of T_4 to T_3 by hepatic homogenates of the rat. As glutathione is the principal endogenous cellular nonprotein thiol (51), this finding suggests that cells possess a cofactor capable of stimulating enzymatic activity. Recently, Leonard and Rosenberg (52) have proposed a model for the enzyme-catalyzed 5'-monodeiodination of T_4. A schematic representation of this model is shown in Figure 2. According to this model the protein structure of the enzyme possesses a thiol group which is consumed during the outer ring reductive deiodination of T_4. For the enzyme to be returned to its active state, a source of reduced sulfhydryl groups, of which glutathione is the most abundant, is necessary. Thus, the sulfhydryl in glutathione is also consumed during a catalytic cycle, and therefore glutathione can be considered a second substrate, reducing the

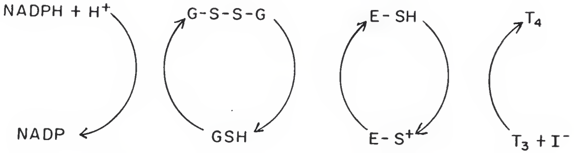


Figure 2. A tentative model of the enzyme-catalyzed reaction which monodeiodinates thyroxine (T_4) to triiodothyronine (T_3).

sulfhydryl moieties of the enzyme, which are oxidized during the formation of T_3 from T_4. Presumably, oxidized glutathione is itself reduced by the flavoprotein, glutathione reductase, which utilizes NADPH (53). Therefore, this model illustrates the importance of a reducing capacity, such as that of the cytosol, in the T_4 5'-monodeiodinating activity of the cell. In addition, since the thiol dependency of the 5'-deiodinase was demonstrated in 1976, studies of the enzyme in vitro have been performed in the presence of sulfhydryl protecting agents.

Specific Aims

A greater understanding of the mechanism by which T_4 is enzymatically monodeiodinated to T_3 by the peripheral tissues has enabled investigators to evaluate more accurately factors capable of modulating this reaction and also to assess whether a change in a thyroid status-dependent physiological function might result from an alteration in the rate of conversion of T_4 to the more potent T_3.

A considerable body of evidence suggests that the thyroid state of an animal plays a permissive role in its ability to respond to β-adrenergic stimulation (for reviews, see references 54 and 55). Studies from this laboratory have demonstrated that acclimation to cold, renal hypertension, and chronic administrations of either estrogen, the antithyroid agent, aminotriazole, or the phosphodiesterase inhibitor, theophylline, are associated with changes in the ability of rats to respond to β-adrenergic stimulation (56-60). Therefore, the possibility exists that a change in thyroid activity of rats under these conditions might be responsible for the observed alteration in β-adrenergic responsiveness. These studies were designed to evaluate the effect of the various physiological and pharmacological states described above on the concentration of T_4 and T_3 in the serum and on in vitro 5'-monodeiodination of T_4 to T_3 by 2,000 x g supernatants of homogenates of liver and kidney. It was not the intent of these studies to answer directly the question of whether a change in these aspects of thyroid hormone activity is the cause of a change in β-adrenergic responsiveness. Rather, they serve to describe whether a change in thyroid activity occurs under these conditions, and in so doing have succeeded in evaluating further those factors capable of regulating this process.

GENERAL METHODS

Treatment regimens, statistical analysis of the data, and details pertinent to each study will be described with the individual experiments. However, the method used to measure the rate of 5'-monodeiodination of T_4 to T_3 by the liver and kidneys, and those to measure the concentration T_3 and T_4 in serum are common to all experiments and will be described here.

In each experiment rats were killed by decapitation between 9 A.M. and 10 A.M. and the trunk blood was collected. The serum was separated and stored at -20°C. The total concentrations of T_3 and T_4 in the sera were later determined by a commercially available radioimmunoassay (RIA) kit (Diagnostic Products Co., Los Angeles, CA). In addition, livers and kidneys were dissected, washed in cold (4°C) 0.15 M potassium phosphate buffer (pH 7.4), blotted, weighed and homogenized in 3 volumes of buffer for 30 sec using a Tekmar SDT homogenizer. The homogenates were centrifuged at 2,000 x g for 20 min at 4°C. The supernatants were used immediately or frozen in liquid N_2 and stored at -65°C for a maximum of 10 days before use.

The method used to measure the rate of conversion of T_4 to T_3 by 2,000 x g supernatants of homogenates of liver or kidney is a modification of that described by Kaplan *et al*. (49). Aliquots of tissue supernatant were incubated with nonradioactive T_4 (Sigma Chemical Co., St. Louis, MO) for 20 or 30 min, and the amount of T_3 generated was measured by RIA. In a typical experiment the following reagents were added in 13 x 100 mm glass culture tubes: (a) 0.15 M potassium phosphate buffer (pH 7.4), an amount sufficient to adjust the final volume to 1 ml; (b) dithiothreitol (Sigma Chemical Co., St. Louis, MO), 100 µl of a solution containing 4.3 mg/ml; (c) 200 µl aliquots of tissue supernatants; (d) nonradioactive T_4, 10 µl of a solution containing 500 µg/ml of 100% ethanol. All tubes were mixed by swirling and placed in an incubator at 37°C. Each assay was performed in duplicate and the reaction was stopped by addition of 2 ml of 95% ethanol to the incubation mixtures. The tubes were vortexed and immediately placed on ice for 30 min. Samples were centrifuged, and the supernatant was stored at -20°C until assayed for T_3 by RIA.

In addition, each assay included two tubes (0 min incubation tubes) that were handled exactly as described, except that 95% ethanol was added to the incubation mixtures before the addition of nonradioactive T_4. The T_3 content of these tubes was considered to represent T_3 contained in the tissue supernatant, that in the commercial T_4 and that apparent from the cross-reaction of T_4 in the T_3 assay. This amount of T_3 was subtracted from the T_3 measured in test samples to derive the amount of T_3 produced due to incubation of the supernatant. Protein concentration was determined by the Biuret method (61), using bovine serum albumin as a standard. The results were calculated for each supernatant by dividing the mean amount of T_3 generated in 20 or 30 min by the amount of protein added.

Dithiothreitol

Dithiothreitol (DTT) was the sulfhydryl protecting agent of choice for use in these experiments, as it is effective at lower concentrations than either mercaptoethanol or glutathione (as a result of its lower oxidation-reduction potential) and it is not readily oxidized in room air (62). The concentration of DTT used was decided as a result of preliminary studies, in which 2,000 x g supernatants of homogenates of liver or kidney from male Sprague-Dawley rats (200-250 g) were incubated as described above except that the concentration of DTT in the incubation mixtures was varied from 0 to 860 µg/ml. The results of this experiment are shown in Figure 3. A concentration of 430 µg/ml was chosen for use in these studies as this concentration achieved maximal production of T_3 after an incubation period of 30 min.

Homogenate Fractions

The 2,000 x g supernatant fractions of homogenates of liver and kidney were chosen for these studies as these fractions are not contaminated with nuclei, cellular debris or whole cells, and they have similar total T_4 5'-monodeiodinase activity (per gram of tissue) as the whole homogenates (42, 63). To ensure that the rate of generation of T_3 from T_4 was proportional to the concentration of this supernatant fraction at the concentration used in these experiments, the rate of this reaction was measured in incubation mixtures as described above except that they contained between 0 and 400 µl aliquots of the 2,000 x g supernatant fraction of homogenates of liver and kidneys from control male rats and DTT at a concentration of 860 µg/ml. The results of these experiments are shown in Figure 4. It is apparent that after an incubation period of 30 min, T_3 production increased progressively with the addition of increasing aliquots of renal supernatant, and increased linearly with the addition of aliquots of hepatic supernatant up to approximately 300 µl. Beyond this volume, there was no further increase in production of T_3 with the addition of increasing volumes of this supernatant.

Figure 3. Effect of various concentrations of dithiothreitol (DTT) on the rate of monodeiodination of T_4 to T_3 by the 2,000 x g supernatants of homogenates of liver (A) and kidney (B). One standard error is set off at each mean.

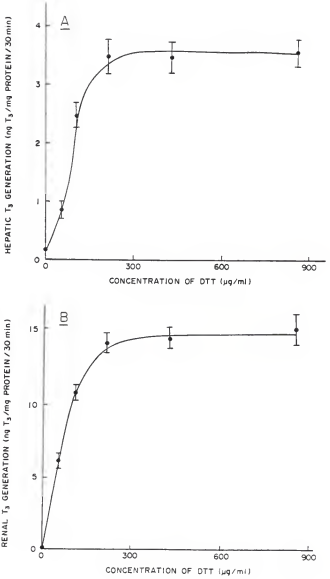
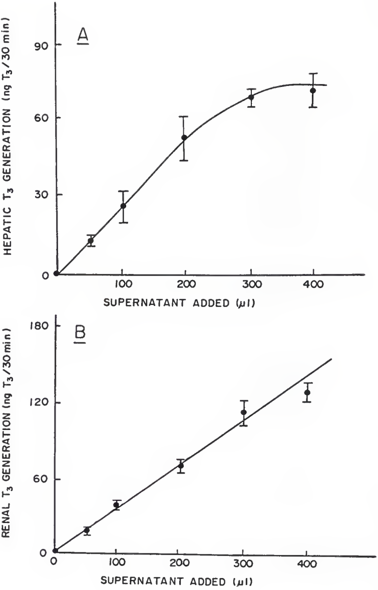

A
HEPATIC T_3 GENERATION (ng T_3/mg PROTEIN/30 min)
0
1
2
3
4
0
300
600
900
CONCENTRATION OF DTT (µg/ml)
B
RENAL T_3 GENERATION (ng T_3/mg PROTEIN/30 min)
0
5
10
15
0
300
600
900
CONCENTRATION OF DTT (µg/ml)

Figure 4. Effect of addition of various volumes of the 2,000 x g supernatant fraction of hepatic (A) and renal (B) homogenates on the rate of monodeiodination of T_4 to T_3. One standard error is set off at each mean.


A
HEPATIC T3 GENERATION (ng T3 / 30 min)
90
60
30
0
100
200
300
400
SUPERNATANT ADDED (µl)
B
RENAL T3 GENERATION (ng T3 / 30 min)
180
120
60
0
0
100
200
300
400
SUPERNATANT ADDED (µl)

Recent evidence has demonstrated that 99% of the T_4 added to incubation mixtures containing microsomes of rat liver is bound both specifically and nonspecifically to this fraction (64). In this study it was noted that the rate of production of T_3 (expressed per mg protein) was greater when lower amounts of microsomes and concentrations of T_4 from 0.3 to 3 μM were used. When higher concentrations of T_4 were used, the rate of generation of T_3 was proportional to the amount of microsomal protein added. In addition, when free instead of total T_4 concentration was used as the substrate concentration for the calculation of K_m, both Lineweaver Burk and Hill plot analyses yielded straight lines, and changes of K_m with different amounts of microsomal protein were not detected. It was concluded that the addition of more binding protein to the incubation mixtures decreases the concentration of free T_4 available for conversion to T_3. It seems likely that the loss of a proportional increase in the rate of production of T_3 with addition of larger aliquots of hepatic supernatants to the incubation mixtures is probably related to this phenomenon. Generation of T_3 by renal supernatants is not affected by the volume of supernatant added to the reaction mixture. A likely explanation for this is that these supernatants contain less than one half as much protein as the hepatic supernatants and hence there is a larger amount of free T_4 available.

The concentration of T_4 (5 μg/ml, 6.4 μM) chosen for these studies was the concentration that yielded the maximal amount of T_3 during incubation of homogenates of liver in the original studies of Visser et al. (40) and Chopra (42). In addition, at the concentration of tissue supernatant used in these studies, this concentration of T_4 appeared to be a saturating concentration, i.e. at these concentrations of supernatant and T_4 the rate of generation of T_3 was dependent on the volume of tissue supernatant present in the incubation mixture. The 2,000 x g supernatant of homogenates will henceforth be referred to as homogenates.

Duration of Incubation

An incubation period of either 20 or 30 minutes was chosen in these studies. The duration of incubation was decided as a result of a preliminary study in which homogenates of either liver or kidney from control male rats were incubated as described above, except that the duration of incubation was varied from 0 to 60 min. The results of these experiments are shown in Figure 5. In agreement with previous studies (40, 65), the generation of T_3 by hepatic homogenates increased linearly with time for the first 30 min and declined thereafter, whereas the generation of T_3 by renal homogenates increased linearly with time for all periods of incubation studied.

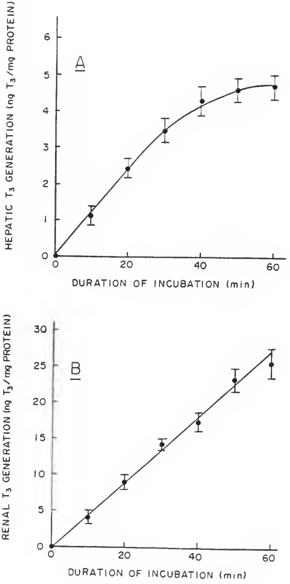
Recovery of 3,5,3'-Triiodothyronine

Use of an in vitro model for measurement of enzymatic activity is dependent on stability of the reaction product in the incubation mixture. No measurable degradation (<10%) of T_3 has been measured after incubation of T_3 with 2,000 x g supernatants of hepatic or renal homogenates and dithiothreitol (5 mM) at 37°C for up to 40 min (49, 63).

Storage of Homogenates

In some of the studies described below the homogenates were frozen in liquid N_2 immediately after preparation and stored at -65°C. Control studies were performed to evaluate any change in T_4 5'-monodeiodinase activity as a result of storage of the homogenates in this manner. Homogenates of liver from five male Sprague-Dawley rats

Figure 5. Effect of duration of incubation on the monodeiodination of T_4 to T_3 by hepatic (A) and renal (B) homogenates. One standard error is set off at each mean.


HEPATIC T_3 GENERATION (ng T_3/mg PROTEIN)
A
0
1
2
3
4
5
6
0
20
40
60
DURATION OF INCUBATION (min)
RENAL T_3 GENERATION (ng T_3/mg PROTEIN)
B
0
5
10
15
20
25
30
0
20
40
60
DURATION OF INCUBATION (min)

(280-320 g) were prepared as described above and each divided into three parts, one of which was assayed immediately for T_4 to T_3 converting activity and the other two were frozen in liquid N_2 and stored at -65°C for either 7 or 32 days. After these periods of time, the T_4 to T_3 converting activity of these homogenates was measured and the results are shown in Table 1. When these data were analyzed by a paired t test (66), it was found that storage of the homogenates at -65°C for either 7 or 32 days did not affect significantly the rate of conversion of T_4 to T_3.

Table 1. Rate of conversion of thyroxine (T_4) to triiodothyronine (T_3) by fresh homogenates of liver and kidney, or by the same homogenates after storage at -65°C for 7 or 32 days.

T_4 to T_3 Converting Activity (ng T_3/mg protein/30 min)	Days of Storage 0	7	32
Hepatic	3.85 ± 0.31*	3.89 ± 0.21	3.86 ± 0.33
Renal	20.33 ± 2.41	21.55 ± 2.33	20.69 ± 3.40

* Mean ± SEM.

SECTION I

EFFECT OF ACCLIMATION TO COLD ON THE RATE OF 5'-MONODEIODINATION OF THYROXINE TO TRIIODOTHYRONINE IN THE RAT

Introduction

Thyroid hormones are considered necessary for survival of rats in the cold (67). Chronic exposure of rats to cold is associated with an increase in several aspects of thyroid function, including an increased rate of uptake of radioactive iodide by the thyroid gland (68), an increased rate of production of T_4 (69, 70), and an increased fecal loss of thyroid hormones (71). In addition, serum concentration of T_4 is normal, while that of T_3 is elevated in rats exposed chronically to 4°C (72). These results indicate that the rates of production, utilization and loss of T_4 increase concommitantly, maintaining a normal concentration of T_4 in the serum of cold-acclimated rats. However, the factors responsible for the elevated concentration of T_3 in the serum of cold-acclimated rats are less certain. It is likely that preferential secretion of T_3 by the thyroid gland contributes to this change (73). However, since extrathyroidal 5'-monodeiodination of T_4 to T_3 accounts for most of the daily production of T_3 in the rat (28), an increase in the rate of this process might also contribute substantially to the elevated concentration of T_3 in the serum of the cold-acclimated rat. Indeed, numerous indirect studies have suggested an increased rate of peripheral deiodination of T_4 to T_3 in cold-acclimated rats (72-76).

As the liver and kidneys are the most active tissues in converting T_4 to T_3 (42), the purpose of this study was first to use the in vitro model to ascertain directly whether the T_4 to T_3 converting activity of homogenates of liver and kidney is enhanced by chronic exposure to cold. Having accomplished this, the mechanism by which cold acclimation stimulates the rate of peripheral deiodination of T_4 was investigated. Several studies have suggested that there is β-adrenergic influence on the deiodination of T_4 in the rat (77, 78) and man (79), and cold acclimation has been shown to increase both catecholamine secretion (80) and the response to β-adrenergic stimulation (56). Also, T_4 to T_3 conversion by hepatic homogenates is increased in hyperthyroid and decreased in hypothyroid rats (81), indicating that the activity of the enzyme is dependent on thyroid status. Furthermore, it has been shown that some of the physiological changes associated with cold acclimation can be mimicked by repeated administration of the β-adrenergic agonist, isoproterenol (ISO), T_4, or a combination of ISO and T_4 (ISO + T_4) to control rats (82-84). Therefore, an attempt was made to mimic the effect of cold acclimation on the rate of 5'-monodeiodination of T_4 by both the liver and the kidney by daily administration of ISO, T_4, or a combination of ISO and T_4, for 20 days. This study was designed to provide insight as to whether an increase in β-adrenergic activity, thyroid state, or a combination of the two, is responsible for the enhanced peripheral conversion of T_4 to T_3 in cold-acclimated rats.

Methods

Thirty male rats of the Blue-Spruce Farms (Sprague-Dawley) strain weighing 200-250 g were housed in groups of three and received tap water and Purina Laboratory Chow ad libitum. The animals were divided randomly into five groups, each consisting of six rats. Group 1 was composed of control animals; group 2 was maintained in a room at 4 ± 1°C; group 3 was injected subcutaneously with 100 µg d,l-isoproterenol (ISO)/kg body weight in oil each day; group 4 was injected with 50 µg l-thyroxine (T_4)/kg b.w./day, s.c.; group 5 was injected with both ISO and T_4 at the same doses given to groups 3 and 4. The ISO and T_4 were purchased from Sigma Chemical Co., St. Louis, MO. The T_4 was prepared by dilution in several drops of 0.1 N NaOH and adjusted to a concentration of 50 µg/ml by addition of physiological saline. Groups 1 and 3 received an injection of the vehicle for T_4 and groups 1 and 4 received an injection of oil (each at a dose of 1 ml/kg b.w./day, s.c.). The animals in groups 1, 3, 4, and 5 were housed in a room maintained at 26 ± 1°C. Illumination in both cold and warm rooms was from 7 A.M. to 7 P.M.

At the end of 20 days, 24 hr after the last injection, the rats were sacrificed and concentrations of T_3 and T_4 in the sera and the rate of T_4 to T_3 conversion by homogenates of liver and kidney were determined as described in the General Methods. Also, the hearts, thyroid glands and interscapular brown fat (IBF) were removed, cleaned of extraneous tissue and weighed on a torsion balance. In this study the homogenates of liver and kidney were frozen at -65°C immediately after preparation for a maximum of 10 days prior to assay. Incubations were performed for 20 min and the results were expressed as a percentage of the mean control value. Statistical analysis was performed by means of the Duncan multiple range test (85), using a Statistical Analysis Package and an Amdahl 470 computer. Significance was set at the 95% confidence limit. In addition, in order to assess the effect of addition of ISO to the incubation mixtures containing homogenates of liver or kidney from control rats, 100 μl of buffer were replaced by an equal volume of buffer containing ISO (10^{-3} M) and the rate of generation of T_3 was determined as described above. These data were analyzed by a paired t test (66) with significance set at the 95% confidence limit.

Results

Table 2 shows the effect of the various treatments on body weights and the ratio of organ to body weight of certain organs. Although the body weights of both the cold-acclimated and T_4-treated rats were less than those of the other groups, the difference from the control group was not significant in either case. The ratio of organ to body weight of liver from the cold-acclimated group was increased significantly above that of the other groups ($p<0.05$). Acclimation to cold, administration of T_4 alone, or in combination with ISO, resulted in an increase in the ratio of kidneys to body weight above that of either the control or ISO-treated group ($p<0.05$). Both cold acclimation and treatment with either ISO or T_4 resulted in significantly larger ratios of heart to body weight than those of the control group ($p<0.01$), while administration of ISO and T_4 together resulted in a ratio of heart to body weight that was significantly larger than that of either the control or T_4-treated group ($p<0.01$, $p<0.05$, respectively). Of all the treatments, cold acclimation was the most effective in increasing the weight ratio of IBF above that of control. Administration of ISO + T_4 resulted in a ratio of IBF to body weight that was significantly less than that of the cold-acclimated group ($p<0.01$), but significantly greater than that of all the other groups ($p<0.01$). The ratio of IBF to body weight from T_4-treated rats was significantly greater than that from control rats ($p<0.01$) and not different from that of the ISO-treated group. The ratio

Table 2. Body weights and organ to body weight ratios of certain organs from the five groups of rats after 20 days of treatment. Values are means ± SEM; 6 animals per group.

Treatment	Body Weight (g)	Liver Wt.(g/ 100 g B.W.)	Kidney Wt.(mg/ 100 g B.W.)	Heart Wt.(mg/ 100 g B.W.)	Thyroid Wt. (mg/100 g B.W.)	Interscapular Brown Fat Wt.(mg/100 g B.W.)
Control	346 ± 7	3.55 ± 0.06	749 ± 21	272 ± 3	7.10 ± 0.52	58 ± 4
Cold-acclimated	319 ± 8	4.16 ± 0.10*	975 ± 48**	345 ± 15+	11.10 ± 0.71*	238 ± 25*
Isoproterenol (100 µg/kg/day, s.c.)	341 ± 13	3.38 ± 0.19	741 ± 18	326 ± 10+	8.27 ± 0.72	91 ± 6
Thyroxine (50 µg/kg/day, s.c)	328 ± 5	3.61 ± 0.06	906 ± 22**	320 ± 8+	6.84 ± 1.00	119 ± 13+
ISO + T_4	346 ± 10	3.48 ± 0.10	833 ± 16**	355 ± 10++	5.92 ± 0.50	178 ± 15*

* Significantly different from all other groups ($p<0.01$).

+ Significantly different from control ($p<0.01$).

++ Significantly different from control and thyroxine-treated groups ($p<0.01$ and $p<0.05$, respectively).

** Significantly different from control and isoproterenol-treated groups ($p<0.05$).

of IBF to body weight from the ISO-treated group was 57% higher than that of the control group, but this difference did not reach statistical significance. Only acclimation to cold resulted in a significant increase in the ratio of thyroid weight to body weight ($p<0.01$) above that of the control group.

The effect of the various treatments on the rate of conversion of T_4 to T_3 is shown in Figure 6. The rates of conversion of T_4 to T_3 by hepatic and renal homogenates from control rats were 4.79 ± 0.16 and 14.23 ± 0.91 ng T_3/mg protein/20 min, respectively. The rate of 5'-deiodination of T_4 by hepatic homogenates from rats acclimated to 4°C for 20 days was 53% higher, while that of renal homogenates was 71% higher than control. The rate of generation of T_3 by hepatic and renal homogenates was not affected significantly by administration of ISO. However, administration of T_4 resulted in a rate of hepatic generation of T_3 which was 297% of control, while the rate of renal generation of T_3 was 222% of control. Administration of ISO in combination with T_4 resulted in a rate of conversion of T_4 to T_3 by homogenates of both hepatic and renal tissue that was not different from that of rats treated with T_4 alone. The addition of ISO to the incubation mixture to achieve a concentration of 10^{-4} M, did not affect significantly the rate of T_3 generation of either control hepatic or renal homogenates (Table 3).

The protein concentrations of hepatic and renal homogenates were not altered significantly by the various treatments (Table 4). Administration of T_4 alone or in combination with ISO resulted in significantly higher concentrations of T_4 in the serum ($p<0.01$). However, only cold acclimation resulted in significant elevation in the concentration of T_3 in serum above that of the control group ($p<0.05$).

Figure 6. Effect of the various treatments on the rate of conversion of T_4 to T_3 by rat hepatic (A) and renal (B) homogenates. Rate of conversion by each group is expressed as a percentage of the control value. All treatments were for 20 days.

* Significantly different from all other groups ($p<0.05$).

** Significantly different from control, cold-acclimated and ISO-treated groups ($p<0.05$).

One standard error is set off at each mean.

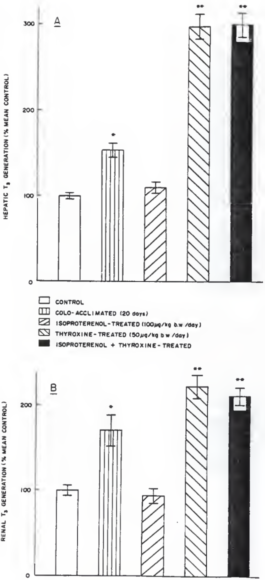

A
HEPATIC T_3 GENERATION (% MEAN CONTROL)
300
200
100
0
*
**
**
CONTROL
COLD-ACCLIMATED (20 days)
ISOPROTERENOL-TREATED (100μg/kg b.w /day)
THYROXINE-TREATED (50μg/kg b.w /day)
ISOPROTERENOL + THYROXINE-TREATED
B
RENAL T_3 GENERATION (% MEAN CONTROL)
200
100
0
*
**
**

Table 3. Effect of isoproterenol (ISO) added to the incubation medium on hepatic and renal T_4 5'-monodeiodinase activity. Values are means ± SEM; 6 animals per group.

T_4 5'-Monodeiodinase Activity*	Control	ISO(10^{-4} M)
Hepatic	100 ± 4	100 ± 7
Renal	100 ± 5	102 ± 6

* Results are expressed as percentage of control.

Table 4. Protein concentrations of homogenates of liver and kidney as well as the concentrations of T_3 and T_4 in the serum of the various groups after 20 days of treatment. Values are means ± SEM; 6 animals per group.

Treatment	Protein conc. of hepatic homogenate (mg/ml)	Protein conc. of renal homogenate (mg/ml)	Serum T_4 concentration (μg/dl)	Serum T_3 concentration (ng/dl)
Control	64 ± 2	28 ± 1	4.2 ± 0.2	93 ± 9
Cold-acclimated	62 ± 2	28 ± 1	4.1 ± 0.2	125 ± 9+
Isoproterenol (100 μg/kg/day, s.c.)	64 ± 3	29 ± 1	4.3 ± 0.1	87 ± 3
Thyroxine (50 μg/kg/day, s.c.)	67 ± 3	27 ± 1	6.3 ± 0.4*	117 ± 12
ISO + T_4	65 ± 2	29 ± 1	5.7 ± 0.3*	112 ± 7

* Significantly different from control, cold-acclimated and isoproterenol-treated groups ($p<0.01$).

+ Significantly different from control and isoproterenol-treated groups ($p<0.05$).

Discussion

Acclimation of rats to air at 6°C has been shown to increase (a) the weight of the heart and IBF; (b) the levels of mitochondrial enzymes in IBF; (c) the resistance to a decrease in body temperature during acute exposure to air at -20°C; and (d) the sensitivity to catecholamines (82-84). It was also demonstrated in these studies that pretreatment of control rats with repeated injections of ISO, T_4, or both ISO and T_4, resulted in physiological changes, some of which parallel those associated with cold acclimation. These include a higher resistance to cooling during exposure to cold and an increased calorigenic response to ISO. Furthermore, administration of either ISO or T_4 increased the weights of heart and IBF. The results of the present study have confirmed the latter findings (Table 2). In addition, when ISO and T_4 were administered together, the ratios of the heart and IBF to body weight were increased even further, but the ratio of IBF to body weight remained significantly less than that of the cold-acclimated group. The inability of the treatment regimens to be as effective as cold acclimation in stimulating an increase in brown fat may reflect its importance for non-shivering thermogenesis in the cold-acclimated rat (86).

In addition, the results of this study demonstrate that the rate of hepatic and renal T_4 to T_3 converting activities is enhanced by chronic exposure to cold. Also, cold acclimation is accompanied by an increase in the concentration of T_3 in the serum, confirming the results of an earlier study (72). Thus, it appears that hepatic and renal deiodination of T_4 may serve as important sources of production of T_3 during cold acclimation. That both hepatic and renal 5'-deiodinases are similarly affected is not surprising as they are considered to be the same enzyme (49).

Although administration of either ISO or T_4 mimicked the effect of chronic exposure to cold on the ratios of heart and IBF to body weight, only the chronic administration of T_4 stimulated the rate of conversion of T_4 to T_3 by hepatic and renal homogenates. In fact, the extent to which this process was stimulated by administration of T_4 was greater than that achieved by cold acclimation. In preliminary studies administration of ISO at doses of 30 and 300 μg/kg b.w./day for 20 days also had no effect on the rate of conversion of T_4 to T_3 by homogenates of either liver or kidney (unpublished observations). Also, the administration of ISO in combination with T_4 failed to increase further the rate of deiodination of T_4 by hepatic and renal homogenates beyond that resulting from administration of T_4 alone, although this treatment did increase significantly the ratios of heart and IBF to body weight above those of the T_4-treated group. These results suggest that the increased β-adrenergic activity of cold-acclimated rats (56, 80) does not play a significant role in stimulating the rate of peripheral T_4 monodeiodination. Rather, it appears that this effect can be accounted for completely by the increase in thyroid activity observed in these animals (68-71).

The present results are surprising in that several studies have suggested that there is β-adrenergic influence on T_4 to T_3 conversion in the rat. Total peripheral deiodination of T_4 _in vivo_ is increased by epinephrine and decreased by reserpine (77). Also, a positive correlation has been reported between total deiodination of T_4 and urinary excretion of catecholamines in cold-exposed rats (76). Furthermore, the rate of conversion of T_4 to T_3 by isolated parenchymal cells of the liver from rats is inhibited by the β-adrenergic antagonist, propranolol

(78). The results of these experiments would suggest that repeated administration of ISO should stimulate that rate of peripheral deiodination of T_4. However, several recent studies contradict such an effect of ISO. Serum concentrations of T_4 and T_3 remain unchanged in cold-acclimated rats either after administration of propranolol or chemical sympathectomy (87), suggesting that β-adrenergically-mediated effects do not influence the rate of conversion of T_4 to T_3 in animals under these conditions. In addition, chronic infusion of low doses of propranolol into control rats did not affect the rate of deiodination of T_4 to T_3 by either hepatic or renal tissue in vitro (88). Furthermore, high concentrations of d,l-, l-, and d-propranolol (the latter isomer lacking β-adrenergic blocking activity) are equipotent in inhibiting the rate of formation of T_3 in isolated renal tubules of rats (89). These results suggest that propranolol inhibits the rate of formation of T_3 directly by its membrane-stabilizing action and not by β-adrenergic blockade. In addition, these investigators failed to demonstrate a change in the rate of deiodination of T_4 when either epinephrine or ISO was added to renal tubules in vitro. Further, the addition of ISO (10^{-4} M) to incubation mixtures containing either hepatic or renal homogenates did not affect the rate of conversion of T_4 to T_3 (Table 3).

Administration of T_4 alone, or in combination with ISO, resulted in a significant increase in the rate of conversion of T_4 to T_3 by hepatic tissue, confirming an earlier study (81). The latter study demonstrated that the rate of 5'-monodeiodination of T_4 by homogenates of liver but not kidney is increased by administration of T_4. However, the results of the present study indicate that renal homogenates also exhibit increased 5'-deiodinase activity when T_4 is administered.

The fourfold greater duration of treatment used in this study may be responsible for the disparity between this and the earlier findings. If this is so, it suggests that the 5'-deiodinase enzyme of the kidney is less sensitive to the effect of T_4 than that of the liver. However, these results support the hypotheses that the T_4 5'-deiodinase enzymes of the liver and kidney are the same and that the enzymatic activity is dependent on thyroid status (49, 81).

Although the present study suggests that the increased thyroid activity of cold-acclimated rats is responsible for the enhanced hepatic and renal 5'-deiodination of T_4, it is possible that other factors might stimulate this process. Indices of adrenal function have suggested that the rate of secretion of glucocorticoids is enhanced by cold exposure (70, 90). However, it is unlikely that corticosteroids play a stimulatory role in this respect, as they have been shown actually to inhibit the rate of peripheral deiodination of T_4 in the rat (91) and man (92). Several studies have suggested that 3,3',5'-triiodothyronine (rT_3) is a potent competitive inhibitor of T_4 to T_3 conversion _in vitro_ (42, 91). It is possible that cold acclimation reduces the formation of rT_3 and hence releases its inhibitory influence on T_3 formation. However, the physiological role of rT_3 in the regulation of this process has been questioned recently (93).

Serum concentrations of T_4 were elevated significantly by administration of either T_4 or ISO + T_4, but only in the cold-acclimated group was the concentration of T_3 in the serum significantly higher than that of the control group. This might seem surprising in light of the considerably higher rates of conversion of T_4 to T_3 by hepatic and renal tissue from the T_4- and ISO + T_4-treated groups. However, this system measures enzymatic activity in vitro under optimal conditions. The limiting factor for the rate of peripheral conversion of T_4 to T_3 in vivo is not known with certainty. It has been suggested to be the concentration of the enzyme (94), concentration of the endogenous cofactor, glutathione (50), or tissue uptake of T_4 (95). The differences cited above suggest that this is an area deserving of further study. In addition, the rate of deiodination of T_3 in vivo, as well as its hepatic conjugation and fecal loss may be altered by the various treatments.

Therefore, the results of this study suggest that the increased thyroid state of cold-acclimated rats stimulates the rate of monodeiodination of T_4 to T_3 by both renal and hepatic tissue. This apparently contributes to the increased concentration of T_3 in the serum of these animals.

Summary

Groups of male rats were administered isoproterenol (ISO), thyroxine (T_4), or both (ISO + T_4) daily for 20 days in an attempt to mimic the effect of cold acclimation on the rate of outer ring deiodination of T_4 to triiodothyronine (T_3) by 2,000 x g supernatants of homogenates of liver and kidney. The rates of hepatic and renal deiodination of T_4 to T_3 in an additional group of rats exposed to 4 ± 1°C for 20 days were 53 and 71% higher, respectively, than control. Administration of ISO (100 μg/kg/day) did not affect the rate of deiodination of T_4 to T_3 by either hepatic or renal tissue. Administration of T_4 (50 μg/kg/day) resulted in rates of hepatic and renal deiodination of T_4 that were 297 and 222% higher, respectively, than control. Administration of ISO + T_4 resulted in a rate of conversion of T_4 to T_3 not different from

that observed when T_4 was administered alone. Serum concentration of T_4 was elevated after administration of both T_4 and ISO + T_4, while serum concentration of T_3 was elevated significantly above that of control only in the cold-acclimated group. These results suggest that the increased rate of 5'-monodeiodination of T_4 by hepatic and renal homogenates from cold-acclimated rats is not a result of increased β-adrenergic activity, but can be accounted for by the increase in thyroid activity observed in these animals.

SECTION II

EFFECT OF 3-AMINO-1,2,4-TRIAZOLE ON THE RATE OF 5'-MONODEIODINATION OF THYROXINE TO TRIIODOTHYRONINE IN THE RAT

Introduction

Aminotriazole (3-amino-1,2,4-triazole, ATZ) is a herbicide which has found widespread use in agriculture (96). It is known to exert a number of physiological effects in mammals. Aminotriazole is reported to protect animals against experimentally-induced tumors, although it is also classified as a carcinogen (97). In addition, ATZ inhibits the enzymes δ-aminolevulinic acid dehydrase (98) and catalase (99), prostaglandin synthesis (100), and the induction of hepatic cytochrome P450 (101).

Of its many physiological effects, its antithyroid activity has received the most attention. Aminotriazole is goitrogenic, most probably as a result of inhibition of thyroid peroxidase (102). However, investigations of the effect of ATZ on peripheral metabolism of thyroid hormones have yielded conflicting results. Administration of ATZ to rats _in vivo_ significantly reduced the rate of deiodination of T_4 by peripheral tissues (103). On the other hand, deiodination of T_4 by homogenates of liver _in vitro_ was enhanced either by addition of ATZ _in vitro_ or by administration of ATZ _in vivo_ prior to sacrifice (104).

The purpose of this study was twofold. First, using the _in vitro_ model it seemed worthwhile to reassess the effect of administration of ATZ _in vivo_ and _in vitro_, at doses similar to those used by the previous investigators (103, 104), on the rate of monodeiodination of T_4 to T_3 by hepatic and renal homogenates. Second, the ability of T_4 to reverse the changes resulting from administration of ATZ in vivo was studied.

Methods

Eighteen male Sprague-Dawley rats from a breeding colony maintained in our animal facility were used. The animals, weighing initially 210-250 g, were housed three per cage in a room maintained at 25 ± 1°C and illuminated from 6 A.M. to 6 P.M. All rats were allowed tap water and finely ground Purina Laboratory Chow ad libitum. The rats were divided randomly into three groups, each containing six rats. The first group served as a control, the second received ATZ (ICN Pharmaceuticals, Inc., Cleveland, OH) mixed thoroughly into their food at a concentration of 0.5 g ATZ/kg of food; while the third group received ATZ as above plus daily subcutaneous administration of 50 µg T_4/kg body weight/day. The T_4 was prepared as described in Section I. The first two groups received a subcutaneous injection of the vehicle for T_4 (1 ml/kg b.w./day). On the last five days of treatment body weight and food intake were measured to determine the amount of ATZ ingested daily.

At the end of 2 weeks, 24 hr after the last injection, the rats were sacrificed and concentrations of T_3 and T_4 in the sera and the rate of T_4 to T_3 conversion by homogenates of liver and kidney were determined as described in the General Methods. In this study the homogenates of liver and kidney were used immediately after preparation, incubations were performed for 30 min, and the results of the incubation were expressed as the mean amount of T_3 generated per mg of protein per 30 min. Statistical analysis of the data was made by means of a one way analysis of variance; comparisons between individual groups were analyzed by a t test using the pooled variance from the analysis of variance (85).

In addition, to assess the effect of the addition of ATZ to the incubation mixture containing hepatic or renal homogenates, 100 µl of buffer were replaced by an equal volume of buffer containing ATZ (10^{-3} or 10^{-1} M) and the rate of generation of T_3 was determined as described above. These data were analyzed by a paired t test (66). Significance for analysis of all data was set at the 95% confidence limit.

Results

Control rats ingested 74 ± 1 g of food/kg b.w./day, while the ATZ- and ATZ + T_4-treated rats ingested 58 ± 2 (SE) and 82 ± 2 g/kg b.w./day respectively. Therefore the amounts of ATZ ingested by the ATZ- and ATZ + T_4-treated groups were 29 ± 1 and 41 ± 1 mg/kg b.w./day respectively. Body weights of rats administered ATZ for 2 weeks were significantly less than control ($p<0.05$), while administration of T_4 in combination with ATZ resulted in body weights not different from control (Table 5). Hepatic and renal weights were significantly reduced by treatment with ATZ, while treatment with ATZ + T_4 resulted in an increase in renal weight ($p<0.01$). The protein concentrations of hepatic homogenates of the ATZ- and ATZ + T_4-treated groups were significantly less than control ($p<0.05$ and $p<0.01$, respectively), while those of the renal homogenates did not differ among the groups.

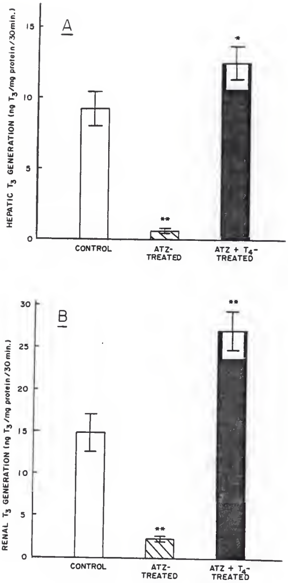
The rate of 5'-monodeiodination of T_4 by hepatic homogenates of the ATZ-treated group was 7% of control, while that of the ATZ + T_4-treated group was 135% of control (Figure 7A). In addition, treatment with ATZ resulted in a rate of renal T_3 generation which was 15% of control, while the rate of renal T_3 generation of the ATZ + T_4-treated rats was 182% of control (Figure 7B). The addition of ATZ to the incubation mixtures to achieve concentrations of 10^{-2} or 10^{-4} M,

Table 5. The effects of treatment with either aminotriazole (ATZ) or aminotriazole plus thyroxine (ATZ + T_4) on body weight, the weights of liver and kidney, and on protein concentrations of hepatic and renal homogenates. Values are means ± SEM; 6 animals per group.

	B.W. (g)	Liver Wt. (g/100 g b.w.)	Kidney Wt. (g/100 g b.w.)	Liver homogenate; protein concentration (mg/ml)	Renal homogenate; protein concentration (mg/ml)
Control	311 ± 6	3.96 ± 0.04	0.79 ± 0.02	71 ± 3	26 ± 1
ATZ-treated	286 ± 9*	3.67 ± 0.09**	0.67 ± 0.01**	63 ± 2*	27 ± 1
ATZ + T_4-treated	291 ± 4	4.14 ± 0.06	0.86 ± 0.01**	54 ± 1**	25 ± 1

* Significantly different from control ($p<0.05$).
** Significantly different from control ($p<0.01$).

Figure 7. Effect of treatment with aminotriazole (ATZ) or aminotriazole plus thyroxine (ATZ + T_4) on the rate of conversion of T_4 to T_3 by hepatic (A) and renal (B) homogenates.
* Significantly different from control ($p<0.05$).
** Significantly different from control ($p<0.01$).
One standard error is set off at each mean.


A
HEPATIC T3 GENERATION (ng T3/mg protein/30 min.)
15
10
5
0
*
**
CONTROL
ATZ-TREATED
ATZ + T4-TREATED
B
RENAL T3 GENERATION (ng T3/mg protein/30 min.)
30
25
20
15
10
5
0
**
**
CONTROL
ATZ-TREATED
ATZ + T4-TREATED

did not affect significantly the rate of generation of T_3 by either control hepatic or renal homogenates (Table 6). Concentrations of both T_3 and T_4 in the serum of the ATZ-treated group were reduced significantly below control, while serum T_4 concentration of the ATZ + T_4-treated group was significantly elevated above control ($p<0.01$) (Table 7). However, serum T_3 concentration of this group was significantly less than control ($p<0.01$).

Discussion

The results of these studies suggest that dietary administration of ATZ for 2 weeks results in a considerable reduction of both renal and hepatic T_4 to T_3 converting activities. In a previous study administration of ATZ _in vivo_ reduced total peripheral thyroid hormone deiodination, as measured by ^{131}I excretion after injection of ^{131}I-labeled T_4 (103). The present study indicates that the reduction in the activity of the outer ring monodeiodinase, which deiodinates T_4, 3,3',5'-triiodothyronine, and probably 3',5'- and 3,3'-diiodothyronine as well (105), is at least partially responsible for these previous findings. Whether the deiodination of other thyroid hormones is altered by administration of ATZ is not known.

However, these findings are not in agreement with those of Galton and Ingbar (104). These investigators showed that adminstration of ATZ _in vivo_ and _in vitro_ increased _in vitro_ T_4 deiodination by hepatic but not renal homogenates of the mouse. In the present study the addition of ATZ to the incubation mixture had no effect on generation of T_3 by either hepatic or renal homogenates. It is most likely that different assay conditions and different species can account for the disparities between the two studies. Incubations in the first study were carried out

Table 6. Effect of aminotriazole (ATZ) added to the incubation medium on hepatic and renal T_4 5'-monodeiodinase activity. Values are means ± SEM; 6 values per group.

T_4 5'-Monodeiodinase Activity*	Control	ATZ (10^{-2} M)	ATZ (10^{-4} M)
Hepatic	100 ± 13	90 ± 9	90 ± 8
Renal	100 ± 15	105 ± 13	106 ± 12

* Results are expressed as percentage of control.

Table 7. Effects of treatment with aminotriazole (ATZ) or aminotriazole plus thyroxine (ATZ + T_4) on serum concentrations of thyroxine (T_4) and triiodothyronine (T_3). Values are means ± SEM.

	No. of Rats	Serum T_4 concentration (μg/dl)	Serum T_3 concentration (ng/dl)
Control	6	4.7 ± 0.2	170 ± 3
ATZ-treated	6	0.9 ± 0.0*	81 ± 2*
ATZ + T_4-treated	6	6.1 ± 0.3*	154 ± 5*

* Significantly different from control ($p<0.01$).

in 100% O_2, while recent studies have shown that anaerobic conditions and reducing agents enhance enzyme activity (65). The conditions of incubation in the present study undoubtedly allowed greater enzyme activity to occur. The lack of an effect when ATZ was added in vitro suggests that the mechanism of action of this compound differs from that of propylthiouracil (PTU), which has been shown to inhibit peripheral 5'-monodeiodination of T_4 at micromolar concentrations (91). It has been suggested that PTU inactivates the enzyme by formation of a disulfide bond (52). If this is the case, it is not surprising that ATZ does not affect 5'-monodeiodination by this mechanism since its molecular structure contains no sulfur.

However, both PTU in an earlier study (106) and ATZ in the present study reduced the rate of peripheral deiodination of T_4 when they were administered to rats in vivo. It is likely that PTU acts, at least partially, by direct inhibition of enzyme activity as discussed, but the mechanism of action of ATZ under these conditions is less certain. Aminotriazole is thought to depress thyroid hormone synthesis by inhibition of the thyroid peroxidase enzyme (102), and it reduces uptake of radioactive iodide by the thyroid gland at a dose similar to that used in this study (107). Furthermore, a state of hypothyroidism reduces 5'-monodeiodinase activity in hepatic and renal homogenates (81). The major factor responsible for defective peripheral T_3 formation in hypothyroidism is a decrease in the activity of the 5'-deiodinase, although a reduction in the concentration of cofactors, glutathione and NADPH, may also contribute (94). A marked hypothyroidism resulted from administration of ATZ in vivo in this study, as indicated by reduced body, hepatic and renal weights, a reduced food intake and reduced serum concentrations of T_4

and T_3. Therefore, it is possible that the effect of ATZ on hepatic and renal T_4 5'-deiodination is indirect and secondary to inhibition of thyroid hormone synthesis.

This suggestion is strengthened by the effect of administration of T_4 in addition to ATZ. Although this group of animals ingested over one third more ATZ than the group receiving ATZ alone, most physiological parameters of thyroid status were returned to control or above control levels. While treatment with ATZ + T_4 resulted in a rate of hepatic and renal generation of T_3 which exceeded that of control, treatment of PTU-treated rats with T_4 failed to return peripheral metabolism of T_4 to normal (108). Although it is possible that ATZ exhibits a direct effect on hepatic and renal deiodination of T_4 by an unknown mechanism, the ability of T_4 to reverse the effects of administration of ATZ suggests that the enzymatic defect is due to hypothyroidism per se. However, the increased rate of T_3 generation by liver and kidneys under these conditions was not sufficient to return serum T_3 concentrations to normal, most likely due to continued suppression of T_3 production of thyroidal origin and loss of its contribution to circulating T_3 concentration. The possibility that an increased rate of hepatic conjugation of thyroid hormones could account for this observation can be ruled out as this has been shown to be actually decreased under these conditions (103). Hence, other compounds which directly block only thyroid hormone synthesis would be expected to reduce peripheral metabolism of T_4 by a similar mechanism.

In addition, the administration of ATZ resulted in a reduction in the protein concentration of hepatic but not renal homogenates. It is uncertain whether ATZ is exhibiting either a direct effect on the liver or an indirect effect resulting from the induced hypothyroidism in this respect. However, recent evidence indicates that both intracellular and secretory protein production is impaired in livers of thyroidectomized rats (109). In addition, part of this deficit persists even after replacement with T_4. The protein concentrations of the hepatic homogenates from the ATZ + T_4-treated group also were significantly less than control, suggesting that at least part of this effect might be a result of hypothyroidism. Such an effect of hypothyroidism has not been demonstrated for the kidney. This is an area which deserves further study.

Studies to determine the minimal dietary dose of ATZ which produces an effect on activity of the thyroid gland have been performed in this laboratory (110). One third of the measures of thyroid function were affected by a dose of 50 ppm ATZ in the food (one tenth of that administered in this study). In future studies it would be worthwhile to establish a dose-response relationship between drug dose and concentrations of T_3 and T_4 in the serum and the rate of T_4 5'-deiodination. Indeed, some of the other effects of ATZ are dose-related. Feinstein et al. (97) have shown that dietary administration of ATZ produces hepatic cancer in mice. This effect has been observed characteristically when the intake of ATZ is at least twentyfold higher than that used in this study. However, nonmalignant adenomatous changes have been observed in the thyroid glands of rats, when the diet contained one fifth the amount of ATZ used in this study (111). This difference is especially apparent when one considers that the accumulation of ^{14}C-labelled ATZ is fourfold greater in the liver than in the thyroid gland (112). It seems likely that the different sensitivities of the two tissues are related to different effects accompanying administration of the compound. It has been proposed that the carcinogenicity of ATZ is at least partially due to inhibition of the catalase enzyme and resultant accumulation of H_2O_2 (97). On the other hand, the effect of ATZ on thyroid hyperplasia has been suggested to be a result of prolonged stimulation of the thyroid gland by thyrotropin, as a consequence of ATZ-dependent depression of thyroid hormone synthesis (111).

Therefore, the results of this study suggest that the action of ATZ on the rate of peripheral deiodination of T_4 to T_3 may be indirect and secondary to inhibition of the synthesis of thyroid hormones and resulting hypothyroidism.

Summary

The effects of administration of aminotriazole (ATZ) both _in vivo_ and _in vitro_ on the rate of outer ring deiodination of thyroxine (T_4) to triiodothyronine (T_3) were evaluated in 2,000 x g supernatants of fresh hepatic and renal homogenates. Administration of ATZ (29 ± 1 mg/kg b.w./day) in the diet for 2 weeks to male rats reduced hepatic and renal T_3 generation to 7 and 15% of control, respectively. This was associated with a significant depression of serum T_3 and T_4 concentrations. Administration of T_4 (50 µg/kg b.w./day) in combination with ATZ increased hepatic and renal T_3 generation to 135 and 182% of control, respectively. The T_4 concentration in the serum of this group was significantly higher, while T_3 concentration was significantly lower, than in control rats. The addition of ATZ (10^{-2} or 10^{-4} M) to the incubation medium _in vitro_ did not affect significantly the T_3 generation by control hepatic and renal homogenates. Therefore, it is likely that the effect of ATZ on the activity of the T_4 5'-monodeiodinase enzyme is indirect and secondary to inhibition of the synthesis of

thyroid hormones and resulting hypothyroidism. The results of the first two sections emphasize the sensitivity of the T_4 to T_3 converting activities of hepatic and renal homogenates to the thyroid state of the animal. Evaluation of the effects of various physiological and pharmacological states on the peripheral 5'-monodeiodination of T_4 should be considered with this in mind.

SECTION III

EFFECT OF THEOPHYLLINE ON THE RATE OF 5'-MONODEIODINATION OF THYROXINE TO TRIIODOTHYRONINE IN THE RAT

Introduction

Theophylline is a methylated xanthine, commonly used as a bronchodilator in the treatment of acute and chronic asthmatic symptoms (113) and in the management and prevention of neonatal apnea (114). At effective concentrations of the drug similar to therapeutic levels in human plasma, theophylline is an antagonist of the actions of prostaglandins (115) and adenosine (116). At higher concentrations, theophylline inhibits phosphodiesterase activity (117), is a benzodiazepine antagonist (118), and modulates translocation of Ca^{++} in skeletal and cardiac muscle (119). As a result of one or more of these actions, numerous effects on the endocrine system have been observed after administration of theophylline. These include an increase in the concentrations of catecholamines, 11-hydroxycorticosteroids and insulin in the plasma (for review, see 114).

Recently, Spindel _et al_. (120) demonstrated that acute administration of high doses of theophylline reduced the concentrations of thyrotropin (TSH), T_4 and T_3 in the serum of rats. These findings suggested the need to assess various aspects of the production of thyroid hormones during administration of theophylline at therapeutically relevant doses. Therefore, the purpose of this study was threefold. First, the effect of prolonged administration of low doses of theophylline on the serum concentrations of T_3 and T_4 in rats and on the rate of in vitro deiodination of T_4 to T_3 by homogenates of their liver and kidneys was assessed. Second, the ability of T_4 to reverse the changes resulting from treatment with theophylline in vivo was also studied. And third, the direct effect of theophylline on the deiodinating process was evaluated by addition of the drug to incubation mixtures containing hepatic or renal homogenates from untreated rats.

Methods

Twenty-four male rats of the Blue-Spruce Farms (Sprague-Dawley) strain, weighing initially 200-250 g were housed 3 per cage in a room maintained at 25 ± 1°C and illuminated from 6 A.M. to 6 P.M. All rats were allowed tap water and finely ground Purina Laboratory Chow ad libitum. The rats were divided randomly into four groups, each consisting of six rats. Group 1 was composed of control animals; group 2 was injected subcutaneously with 25 μg l-thyroxine/kg body weight each day; group 3 received theophylline (2.5 g/kg of food) mixed thoroughly into their food; while group 4 was injected with T_4 and received theophylline in the food in a similar manner to groups 2 and 3. Theophylline was purchased from ICN Pharmaceuticals, Inc., Cleveland, OH. The T_4 was prepared as described in Section I, except that a concentration of 25 rather than 50 μg/ml was used. Groups 1 and 3 received a subcutaneous injection of the vehicle for T_4 (1 ml/kg b.w./day). On the last five days of treatment body weights and food intakes for each cage were measured to determine the amount of theophylline ingested daily.

At the end of two weeks, 24 hr after the last injection of T_4, the rats were sacrificed, and the concentrations of T_3 and T_4 in the sera and the rate of conversion of T_4 to T_3 by homogenates of liver and kidney were determined as described in the General Methods. In addition, the thyroid glands were removed, cleaned of extraneous tissue and weighed on a torsion balance. The concentration of theophylline in the sera was also determined by an enzymatic immunoassay technique (EMIT, Sylva Corporation, Palo Alto, CA). In this study the homogenates of liver and kidney were frozen at -65°C immediately after preparation for a maximum of 10 days prior to assay. Incubations were performed for 20 min and the results were expressed as a percentage of the mean control value. Statistical analysis of the data was performed by means of a two-way analysis of variance for a factorially designed experiment (121). Comparison between individual groups was made by a t test using the pooled variance from the analysis of variance (85).

To assess the effect of the addition of theophylline to the incubation mixtures containing control hepatic and renal homogenates, the rate of generation of T_3 was determined in the presence of theophylline (10^{-2} or 10^{-4} M). These data were analyzed by a paired t test (66). Significance for analysis of all data was set at the 95% confidence limit.

Results

Control rats ingested 72 ± 1 (SE) g of food/kg b.w./day during the last five days of the experiment, while the T_4-treated animals ingested 77 ± 1 g/kg/day ($p<0.01$). The food intakes of the theophylline- and theophylline + T_4-treated groups were also significantly greater than control ($p<0.05$ and $p<0.01$, respectively), but were not significantly different from each other (76 ± 1 and 77 ± 1 g/kg/day, respectively). Therefore, the data for the latter two groups were combined to calculate the amount of theophylline ingested, 191 ± 5 mg/kg b.w./day. This dose of theophylline resulted in a marked elevation in the concentration of theophylline in the serum (Table 8). In addition, administration of theophylline alone or in combination with T_4 resulted in a reduction in the concentration of T_4 in the serum below that of control ($p<0.01$ and $p<0.05$, respectively), while serum concentrations of T_3 were not altered by the various treatments.

The body weights and ratios of liver to body weight were not affected by the various treatments, while the ratio of kidney to body weight was increased significantly above that of control by administration of T_4 alone or in combination with theophylline ($p<0.01$) (Table 9). Although 47% larger, the ratio of thyroid to body weight of the theophylline-treated group (4.02 ± 0.76 mg/100 g b.w.) was not statistically different from that of control (2.73 ± 0.34 mg/100 g b.w.). The ratios of thyroid to body weight of the groups receiving T_4 alone or in combination with theophylline were 2.42 ± 0.25 and 3.10 ± 0.32 mg/100 g b.w., respectively. The protein concentrations of the 2,000 x g supernatant of hepatic or renal homogenates did not differ significantly among the groups.

The effect of the various treatments on the rate of conversion of T_4 to T_3 is shown in Figure 8. The rate of conversion of T_4 to T_3 by hepatic and renal homogenates from control rats was 5.88 ± 0.26 and 19.54 ± 0.84 ng T_3/mg protein/20 min, respectively. Administration of T_4 for 14 days resulted in a rate of hepatic generation of T_3 which was 219% of control, while the rate of renal T_3 generation was 159% of control ($p<0.01$). The T_4 5'-monodeiodinase activities of hepatic and renal homogenates were reduced to 67 and 76% of the mean

Table 8. Effect of administration of thyroxine (T_4), theophylline (THEO), or a combination of the two, for 2 weeks on the concentrations of T_4, triiodothyronine (T_3) or THEO in the serum. Values are means ± SEM; 6 animals per group.

Treatment	Serum T_4 Concentration (μg/dl)	Serum T_3 Concentration (ng/dl)	Serum THEO Concentration (μg/ml)
Control	5.8 ± 0.1	123 ± 10	Undetectable (<2.5)
Thyroxine (25 μg/kg b.w./day)	6.1 ± 0.4	116 ± 13	Undetectable (<2.5)
Theophylline (191 mg/kg b.w./day)	4.4 ± 0.4**	124 ± 7	29.7 ± 4.6
T_4 + THEO	4.7 ± 0.3*	113 ± 6	27.3 ± 4.8

** Significantly different from control ($p<0.01$)

* Significantly different from control ($p<0.05$)

Table 9. Effect of administration of thyroxine (T_4), theophylline (THEO) or a combination of the two, on the body weight and the organ to body weight ratios of the liver and kidneys, as well as the protein concentrations of 2000 x g supernatants of homogenates of liver and kidney after 14 days of treatment. Values are means ± SEM; 6 animals per group.

Treatment	B.W. (g)	Liver Wt. (g/ 100 g B.W.)	Kidney Wt. (mg/ 100 g B.W.)	Protein conc. of hepatic homogenate (mg/ml)	Protein conc. of renal homogenate (mg/ml)
Control	341 ± 8	3.92 ± 0.08	790 ± 30	66 ± 2	25 ± 1
Thyroxine (25 µg/kg b.w./day)	325 ± 8	4.06 ± 0.09	917 ± 34**	62 ± 4	24 ± 1
Theophylline (191 mg/kg b.w./day)	324 ± 5	4.11 ± 0.06	872 ± 27	62 ± 2	23 ± 1
T_4 + THEO	331 ± 5	4.01 ± 0.09	926 ± 18**	66 ± 2	23 ± 1

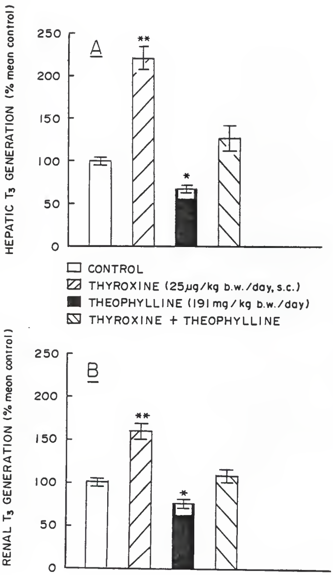
** Significantly different from control ($p<0.01$)

Figure 8. Effect of treatment with thyroxine (T_4), theophylline (THEO), or a combination of the two, on the rate of conversion of T_4 to T_3 by hepatic (A) and renal (B) homogenates. Rate of conversion of each group is expressed as a percentage of the mean control value. All treatments were for 14 days.

* Significantly different from control and T_4 + THEO-treated groups ($p<0.05$ and $p<0.01$, respectively).

** Significantly different from all other groups ($p<0.01$).

One standard error is set off at each mean.


HEPATIC T_3 GENERATION (% mean control)
A
250
200
150
100
50
0
**
*
CONTROL
THYROXINE (25µg/kg b.w./day, s.c.)
THEOPHYLLINE (191 mg/kg b.w./day)
THYROXINE + THEOPHYLLINE
RENAL T_3 GENERATION (% mean control)
B
250
200
150
100
50
0
**
*

control levels by administration of theophylline ($p<0.05$). When both T_4 and theophylline were administered, the rate of generation of T_3 by hepatic and renal homogenates was similar to that of control homogenates. A significant interaction was observed between T_4 and theophylline treatments in affecting the rate of conversion of T_4 to T_3 by homogenates of liver and kidney ($p<0.01$ and $p<0.05$, respectively).

As a three- to fourfold variation in the concentration of theophylline was observed in the serum of animals receiving theophylline alone or in combination with T_4, it seemed worthwhile to investigate whether a relationship existed between the logarithm of the concentration of theophylline in the serum of each animal and any other parameter measured. A significant negative correlation between the rate of generation of hepatic and renal homogenates and the logarithm of the concentration of theophylline in the serum was observed in animals receiving theophylline alone ($p<0.05$) (Figure 9).

The presence of theophylline (10^{-2} and 10^{-3} M) in the incubation mixtures inhibited significantly the rate of T_3 generation by hepatic homogenates ($p<0.01$), while T_4 5'-deiodinase activity of renal homogenates was inhibited by theophylline only at 10^{-2} M ($p<0.01$) (Figure 10). Theophylline (10^{-4} M) did not affect significantly the rate of conversion of T_4 to T_3 by either hepatic or renal homogenates.

Discussion

Administration of theophylline in the diet is an effective means of elevating the concentration of the drug in the serum of rats, a daily intake of 191 mg of theophylline/kg b.w./day achieving a mean concentration of 28.5 µg/ml of serum. This is a concentration higher than that

Figure 9. Relationship between serum concentration of theophylline and the rate of T_4 to T_3 conversion by hepatic (A) and renal (B) homogenates from rats administered theophylline alone or in combination with T_4 for 14 days. Equations of the lines for the theophylline-treated group are shown separately in each figure.
The upper bar respresents the mean ± SEM for hepatic (A) and renal (B) T_3 generation (ng T_3/mg protein/20 min) by the T_4-treated group and the lower bar represents those by the control group. The bars are drawn for purpose of reference only.

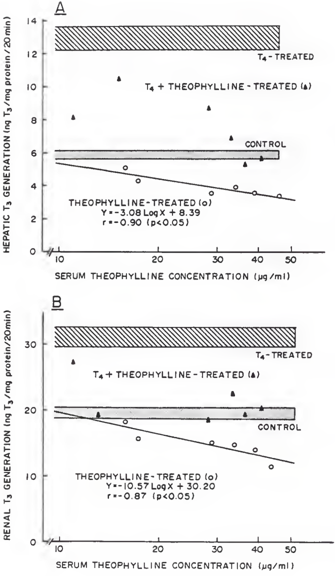
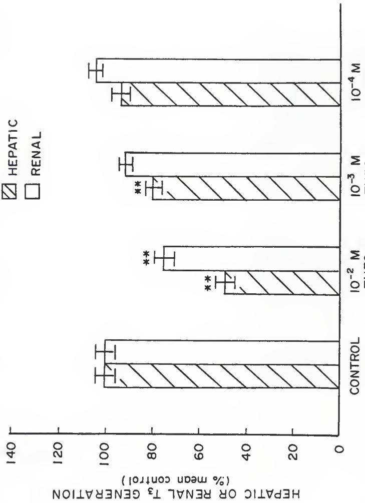

A
HEPATIC T3 GENERATION (ng T3/mg protein/20min)
14
12
10
8
6
4
2
0
T4-TREATED
T4 + THEOPHYLLINE-TREATED (▲)
CONTROL
THEOPHYLLINE-TREATED (o)
Y = -3.08 Log X + 8.39
r = -0.90 (p<0.05)
10
20
30
40
50
SERUM THEOPHYLLINE CONCENTRATION (μg/ml)
B
RENAL T3 GENERATION (ng T3/mg protein/20min)
30
20
10
0
T4-TREATED
T4 + THEOPHYLLINE-TREATED (▲)
CONTROL
THEOPHYLLINE-TREATED (o)
Y = -10.57 Log X + 30.20
r = -0.87 (p<0.05)
10
20
30
40
50
SERUM THEOPHYLLINE CONCENTRATION (μg/ml)

Figure 10. Effect of theophylline (THEO) added to the incubation medium on hepatic and renal T_4 5'-monodeiodinase activity. Values are expressed as a percentage of the mean control value; 6 values per group.

** Significantly different from control ($p<0.01$).

One standard error is set off at each mean.


HEPATIC
RENAL
HEPATIC OR RENAL T3 GENERATION
(% mean control)
140
120
100
80
60
40
20
0
CONTROL
10-2 M THEO
10-3 M THEO
10-4 M THEO
**
**
**

considered within the safe therapeutic range for humans (5 to 20 μg/ml). Within this range, the therapeutic effects of theophylline are proportional to the logarithm of the serum concentration (122), and above it adverse effects such as vomiting and diarrhea are observed (113). Despite the relatively high concentration of theophylline achieved in the present study, similar adverse effects were not observed. In fact, the food intake of the theophylline-treated group was significantly greater than that of the control group, possibly secondary to the increase in basal metabolic rate which is associated with ingestion of methyl xanthines (123). It appears that rats are more resistant to the adverse side effects of high serum concentrations of theophylline than are humans.

A threefold variation in the levels of theophylline in the serum (range 15.9 to 43.8 μg/ml) was observed after administration of the drug in the food, a surprising finding in light of the relatively constant food intake and growth rate of this group. Although individual differences in the absorption of theophylline from the gastrointestinal tract cannot be ruled out, it seems more likely that intersubject variation in the rate of metabolism of the drug is responsible for the variation in the serum levels. Most of the clearance of theophylline in the rat is achieved by biotransformation by the liver microsomal system and is therefore subject to induction or inhibition by a variety of substrates (124). Indeed, the considerable range in the clearance of theophylline in humans (8 to 200 ml/kg/hr) has been attributed, at least in part, to the effects of different genetic and environmental factors on its metabolism (125, 126). That genetically inbred male rats, maintained under identical controlled conditions, might also exhibit a considerable variation in metabolism is intriguing.

Administration of T_4 in combination with theophylline resulted in a serum concentration of theophylline, which was not different from that achieved by administration of theophylline alone, although again there was considerable variation (range 11.0 to 40.5 µg/ml). This is surprising, as it is well documented that the activity of many hepatic microsomal drug-metabolizing enzymes is dependent on the thyroid state of the animal (127, 128). Therefore, it might have been expected that administration of T_4, at a dose sufficient to stimulate considerably T_4 5'-deiodinase activity in control animals, would increase the metabolism of theophylline and hence result in a decreased serum concentration. It is possible that although one or more of the pathways of theophylline metabolism were increased by administration of T_4, this was not reflected by a significant change in the concentration of the drug in the serum. In addition, it is possible that the administration of this dose of T_4, which was sufficient to return T_4 to T_3 converting activity but not serum T_4 concentration to control levels, did not change the thyroid state of the theophylline-treated animals sufficiently for a change in the metabolism of theophylline to be stimulated. In light of its clinical importance, this is an area deserving of further study.

The results of the present study demonstrate that dietary administration of theophylline for 2 weeks reduces significantly both hepatic and renal T_4 to T_3 converting activities. Indeed, the variation in serum levels of theophylline achieved by the treatment presented the opportunity for a significant, negative relationship to be demonstrated between the rate of conversion of T_4 to T_3 by hepatic and renal homogenates and the logarithm of the concentration of the drug in the serum. It appears that administration of theophylline to achieve levels greater than 15 to 20 μg/ml of serum was most effective in eliciting these changes. No other parameter measured correlated significantly with the logarithm of the concentration of theophylline in the serum.

Using a therapeutically more relevant regimen, the results of the present study confirm the findings of Spindel et al. (120), who demonstrated that acute administration of theophylline reduces the serum concentration of T_4. As this change was preceded by a decrease in the concentration of TSH in the serum, these investigators suggested that the effect of theophylline was mediated by a reduction in the stimulation of thyroid hormone secretion by TSH. If such a reduction in serum concentration of TSH persists with long-term administration of theophylline, as occurred with caffeine (120), the goitrogenic effect of theophylline, observed in this and previous studies (129), probably results from some factor other than hypersecretion of TSH. A direct effect of theophylline on the thyroid gland or an increase in the sensitivity of the gland to TSH might be possible mediators. Nevertheless, the results of the present study suggest that the reduced concentration of T_4 in the serum of theophylline-treated rats is not due to an increased metabolism of T_4, although increased fecal loss of T_4, accompanying the increased food consumption and fecal bulk of these animals, might at least be partly responsible (71).

However, in disagreement with the study of Spindel et al., the serum concentration of T_3 in the present study was unaffected by administration of theophylline. This is a difficult finding to explain in light of the reduced serum concentration of T_4 and depression of peripheral T_4 to T_3 converting activity. However, it is possible that preferential secretion of T_3 by the thyroid gland, as suggested previously (130), or a reduction in hepatic conjugation of T_3 might contribute. However, changes in the concentration of T_3 in the serum with different treatments are difficult to predict. A recent study demonstrated that the serum concentrations of T_3 remained at control levels for over 4 weeks postthyroidectomy, even though the serum concentration of T_4 was undetectable and the thyroid status-dependent T_4 5'-deiodinase activity was considerably depressed (81). The latter findings suggest that a minimal supply of T_4 (presumably of endogenous or dietary origin) and a very low level of peripheral T_4 to T_3 converting activity can maintain serum concentrations of T_3 at control levels. That the serum concentration of T_3, the more potent of the thyroid hormones, is unchanged after thyroidectomy and yet the animal is physiologically hypothyroid is an intriguing problem and will be discussed further in the General Discussion.

The mechanism by which theophylline inhibits the conversion of T_4 to T_3 by hepatic and renal homogenates is uncertain. The results of the present study indicate that the presence of high concentrations of theophylline in the incubation mixture inhibits the T_4 5'-deiodinase activities of hepatic and renal homogenates, indicating that theophylline exerts a direct effect on the enzyme albeit at a concentration three orders of magnitude higher than that required for inhibition of either hepatic or renal T_4 to T_3 conversion by propylthiouracil or iopanoic acid (49, 52). The nature of the inhibition of enzymatic activity by the addition of theophylline in vitro cannot be elucidated by these results and awaits further study. Although local concentrations of the drug in vivo may reach those required for direct inhibition of the enzyme, the concentrations of theophylline in the serum in this study (0.9 to 2.4 x

10^{-4} M) were not sufficient to exert a direct effect on the T_4 5'-deiodinase. However, this possibility will remain unanswered until local concentrations of the drug can be ascertained.

It is also possible that the decrease in the serum concentration of T_4 resulting from administration of theophylline has resulted in a state of hypothyroidism. As the activity of the T_4 5'-deiodinases of the liver and kidney is dependent on the thyroid state of the animal (81), a hypothyroid state would be reflected in a reduced T_4 5'-deiodinase activity. Indeed, this appears to be the case, although other physiological parameters, which are responsive to thyroid state, body weight, food intake, or the ratios of liver or kidney to body weight, do not suggest a significant state of hypothyroidism. Either the state of hypothyroidism is so slight that it is not reflected by significant changes in these physiological parameters, or other effects of the administration of theophylline are compensating for any changes due to hypothyroidism. Indeed, the suggestion that the effect of theophylline on hepatic and renal T_4 5'-deiodinase activity is indirect and secondary to suppression of thyroid hormone synthesis is strengthened by the effect of administration of T_4 in addition to theophylline. This treatment regimen returned T_4 to T_3 converting activities to control levels. Within this group of rats, those with the lowest T_4 to T_3 converting activities tended to have higher levels of theophylline in the serum, although a significant relationship was not reached (Figure 9).

Therefore, the results of this study suggest that administration of low doses of theophylline reduce the concentration of T_4 in the serum and also reduce the rate of conversion of T_4 to T_3 by hepatic and renal homogenates. Although it is more likely that the latter is indirect and secondary to inhibition of thyroid hormone secretion, it is also possible that a direct effect of theophylline on the T_4 5'-deiodinase enzyme may contribute.

Summary

The effect of administration of theophylline (both in vivo and in vitro), thyroxine (T_4), or a combination of the two, for 14 days on the rate of 5'-monodeiodination of T_4 to triiodothyronine (T_3) was evaluated in 2,000 x g supernatants of hepatic and renal homogenates. Administration of T_4 (25 µg/kg b.w./day) for 2 weeks to male rats stimulated the rate of hepatic and renal T_3 generation to 219 and 159% of control, respectively. Adminstration of theophylline (191 mg/kg b.w./day) increased the serum concentration of theophylline to 28.5 µg/ml and reduced the rate of hepatic and renal T_4 to T_3 converting activity to 67 and 76% of the mean control values. Administration of a combination of T_4 and theophylline returned T_4 5'-deiodinase activities to control levels. Administration of theophylline alone or in combination with T_4 reduced the concentration of T_4 in the serum, while that of T_3 was unaffected by the various treatments. The presence of theophylline (10^{-2} and 10^{-3} M) in the incubation mixtures containing hepatic homogenates inhibited T_4 5'-deiodinase activity, while activity of renal homogenates was inhibited only by theophylline at 10^{-2} M. Although it is most likely that the effect of theophylline on the activity of the T_4 5'-deiodinase enzyme is indirect and secondary to the inhibition of thyroid hormone secretion, it is also possible that a direct effect of theophylline on the T_4 5'-deiodinase enzyme may contribute.

In this section, the sensitivity of the peripheral T_4 to T_3 converting system to the thyroid state of the animal has been again demonstrated. In addition, the use of the in vitro model has now allowed investigation of the direct effects of isoproterenol, aminotriazole and theophylline on the activity of the T_4 5'-monodeiodinase enzyme. However, it is apparent that caution should be exercised in correlating in vitro deiodination of thyroid hormones with in vivo hormone metabolism and serum hormone concentrations.

SECTION IV

EFFECT OF ESTROGEN AND THYROXINE ON THE RATE OF 5'-MONODEIODINATION OF THYROXINE TO TRIIODOTHYRONINE IN THE RAT

Introduction

Studies to determine the effects of administration of estrogen on thyroid function have yielded conflicting results. This is probably a result of varying doses and durations of treatment, differences in age and sex as well as the gonadal integrity of the animals. Assessment of the thyroid function of rats chronically treated with estrogen is of some importance, since certain physiological parameters which are known to be affected by the thyroid state of the animal are also altered by administration of estrogen. For example, prolonged administration of estradiol benzoate to intact female rats reduced their response to the β-adrenergic agonist, isoproterenol (131, 132). Thus, the possibility exists that the effect of estrogen on β-adrenergic responsiveness is indirect and secondary to reduced thyroid function.

Therefore, the purpose of this study was twofold. First, it seemed worthwhile to investigate the effect of the same dose of estradiol benzoate (EB) as used in previous studies on both the concentrations of T_3 and T_4 in the serum and the rate of conversion of T_4 to T_3 by the liver and kidneys. The second objective was to determine whether administration of T_4 could return a deficit in any of the these parameters to normal.

Methods

Twenty-four female rats of the Blue-Spruce Farms (Sprague-Dawley) strain weighing 180-200 g were housed in groups of three in stainless steel cages in a room maintained at 25 ± 1°C and lighted from 6 A.M. to 6 P.M. Purina Laboratory Chow and tap water were provided *ad libitum* to all animals.

The animals were divided randomly into four groups, each consisting of six rats. Group 1 served as a control, and was implanted with a 10 mm length of empty Silastic tubing (0.419 mm wall thickness, No. 602-265, Dow Corning, Midland, MI) in the region between the shoulder blades; group 2 was implanted with a 10 mm length of empty silastic tubing in a similar manner to group 1, and was injected subcutaneously with 50 µg 1-thyroxine (T_4)/kg body weight each day; group 3 was implanted with a 10 mm length of similar, but sealed and weighed Silastic tubing containing crystalline β-estradiol-3-benzoate (EB, Sigma Chemical Co., St. Louis, MO) between the shoulder blades; and group 4 was injected with T_4 at a dose similar to that of group 2 and was implanted with Silastic tubing containing EB in a manner similar to group 3. The T_4 was prepared as described in Section I. Groups 1 and 3 received an injection of the vehicle for T_4 (1 ml/kg b.w./day, s.c.). At the end of the experiment the tubes were removed, cleaned of adhering tissue and placed in a vacuum dessicator for 72 hr. Drug dose was based on mean weight loss of each tube and mean body weight during the period of administration. Previous studies have indicated that this method of steroid administration provides a reliable means of achieving relatively constant drug release for periods up to 3 months (131, 132). Since chronic administration of estrogen has been shown either to reduce (133) or have no effect on (134) the food intake of rats and a reduction in food intake is associated with a fall in the rate of conversion of T_4 to T_3 (63), the body weights and food intakes of the various groups were measured each day during the last week of treatment.

At the end of 35 days, 24 hr after the last injection, the rats were sacrificed and concentrations of T_3 and T_4 in the sera and the rate of conversion of T_4 to T_3 by homogenates of liver and kidney were determined as described in the General Methods. In addition, the thyroid glands were removed, cleaned of extraneous tissue and weighed on a torsion balance. Also, the concentration of estradiol in the sera of five rats from each group was determined by radioimmunoassay (135). In this study the homogenates were frozen at -65°C immediately after preparation for a maximum of 10 days prior to assay. Incubations were performed for 30 min and the results were expressed as a percentage of the mean control value. Statistical analysis of the data was performed by means of a two-way analysis of variance for a factorially designed experiment (121). Comparison between individual groups was made by a t test using the pooled variance from the analysis of variance (85). Significance was set at the 95% confidence limit.

Results

The average loss of EB from the tubes of group 3 was calculated to be 12.7 ± 0.6 (SE) μg/day or 58.2 ± 2.7 μg/kg b.w./day, while the loss of EB from the tubes implanted in group 4 was 11.8 ± 0.6 μg/day or 55.1 ± 1.9 μg/kg b.w./day. As the amounts of EB administered to these groups were not significantly different, these data were combined. However, the concentration of estradiol achieved in the serum of the group receiving both T_4 and EB was significantly less ($p<0.05$) than that in the serum of the group receiving EB alone (Table 10). The concentrations of estradiol in the sera of the control and T_4-treated groups were within the range observed in normal cycling female rats (136). A significant interaction was observed between the effects of treatment with T_4 and EB on the concentration of estradiol in the serum ($p<0.01$).

Administration of T_4 alone resulted in a significantly higher concentration of T_4 ($p<0.01$), while administration of EB alone resulted in a significantly lower concentration of T_4 ($p<0.05$) than in the serum of control animals (Table 10). When T_4 and EB were administered together, the concentration of T_4 was significantly less than that in the sera of either the control or T_4-treated groups ($p<0.01$). Therefore, a significant interaction was observed between T_4 and EB treatments in affecting the serum concentration of T_4 ($p<0.01$). Administration of EB and T_4 together resulted in a concentration of T_3 which was significantly higher than that in the serum of the EB-treated group ($p<0.05$). An enlargement of the thyroid gland was observed in those animals treated with EB ($p<0.01$).

The ratios of organ to body weight of the liver and the kidneys were similarly affected by the various treatments, while the body weights did not differ among the groups (Table 11). Administration of T_4 resulted in an increase in the ratio of liver and kidney to body weight above that of the control group ($p<0.05$ and $p<0.01$, respectively). Administration of EB resulted in significantly larger ratios of liver and kidney to body weight than those of either the control or T_4-treated groups ($p<0.05$), while administration of T_4 and EB together resulted in ratios of these organs to body weight which were significantly greater than those of control, T_4-treated or EB-treated groups ($p<0.01$). No evidence for an

Table 10. The effect of administration of either thyroxine (T_4), estradiol benzoate (EB), or a a combination of the two, for 35 days on the concentration of T_3, T_4 and estradiol in the serum and on the ratio of thyroid to body weight. Values are means ± SEM.

Treatment	Serum T_4 concentration (μg/dl)	Serum T_3 concentration (ng/dl)	Serum estradiol concentration (pg/ml)	Thyroid Wt. (mg/ 100 g B.W.)
Control	3.8 ± 0.1 (6)+	121 ± 8 (6)	11 ± 1 (5)	8.71 ± 0.21 (6)
Thyroxine (50 μg/kg/day, s.c.)	4.9 ± 0.2 (6)*	127 ± 10 (6)	9 ± 2 (5)	9.23 ± 0.32 (6)
Estradiol Benzoate (57 μg/kg/day)	3.1 ± 0.2 (6)*	112 ± 12 (6)	205 ± 18 (5)**	14.06 ± 0.38 (6)**
EB + T_4	2.6 ± 0.2 (6)**	146 ± 7 (6)++	134 ± 12 (5)++	9.99 ± 0.40 (6)

( )+ Number of rats in each group.
* Significantly different from control ($p<0.05$).
** Significantly different from control ($p<0.01$).
++ Significantly different from estradiol benzoate-treated group ($p<0.05$).

Table 11. The effect of administration of either thyroxine (T_4), estradiol benzoate (EB), or a combination of the two, for 35 days on body weight, the ratios of liver and kidney to body weight, and the protein concentrations of hepatic and renal homogenates. There are six rats in each group. Values are means ± SEM.

Treatment	B.W. (g)	Liver Wt. (g/100 g B.W.)	Kidney Wt. (mg/100 g B.W.)	Hepatic Homogenates; protein concentration (mg/ml)	Renal Homogenates; protein concentration (mg/ml)
Control	248 ± 2	3.09 ± 0.04	667 ± 17	69 ± 2	24 ± 1
Thyroxine (50 µg/kg/day, s.c.)	245 ± 1	3.43 ± 0.03*	736 ± 13**	69 ± 2	25 ± 1
Estradiol Benzoate (57 µg/kg/day)	239 ± 4	3.98 ± 0.10+	782 ± 6+	57 ± 2**	27 ± 1*
EB + T_4	237 ± 6	4.36 ± 0.10 ++	890 ± 19++	57 ± 2**	26 ± 1

* Significantly different from control ($p<0.05$).

** Significantly different from control ($p<0.01$).

+ Significantly different from control and thyroxine-treated groups ($p<0.05$).

++ Significantly different from control, thyroxine-treated, and estradiol benzoate-treated groups ($p<0.01$).

interaction between T_4 and EB treatments was found in this respect. The protein concentration of the hepatic homogenates of the EB- or EB + T_4-treated groups was significantly less than that of the control group ($p<0.01$), while administration of EB increased the protein concentration of the renal homogenate above that of the control group ($p<0.05$). The food intakes of either the T_4- or EB-treated group (69.7 ± 3.4 and 64.5 ± 3.4 g food/kg b.w./rat/day, respectively) did not differ from that of the control group (63.4 ± 1.1 g/kg b.w./rat/day). However, the food intake of the T_4 + EB-treated group (78.6 ± 2.4 g/kg b.w./rat/day) was significantly greater than that of either the control or EB-treated groups ($p<0.01$).

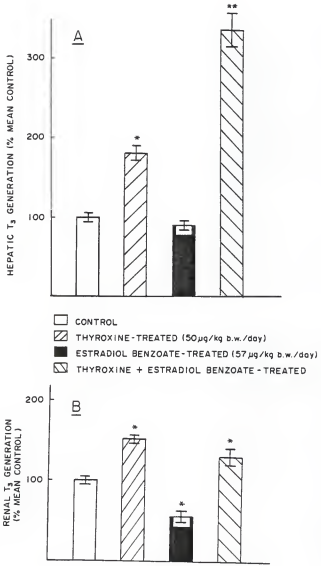
The effect of the various treatments on the rate of conversion of T_4 to T_3 is shown in Figure 11. The rates of conversion of T_4 to T_3 by hepatic and renal homogenates from control rats were 6.33 ± 0.32 and 18.93 ± 0.90 ng T_3/mg protein/30 min, respectively. Administration of T_4 for 35 days resulted in a rate of hepatic generation of T_3 which was 180% of control, while the rate of renal generation was 152% of control ($p<0.01$). The rate of T_4 5'-deiodination by hepatic homogenates from rats chronically treated with EB was 89% of control (not significantly different), while that of renal homogenates was 56% of control ($p<0.01$). Administration of T_4 in combination with EB resulted in a rate of conversion of T_4 to T_3 by hepatic homogenates, which was 334% of control and significantly different from control, T_4-treated and EB-treated groups ($p<0.01$). The rate of renal generation of T_3 by this group of rats was 129% of control ($p<0.01$), but was significantly less than that achieved by administration of T_4 alone ($p<0.05$). A significant interaction was observed between T_4 and EB treatments in affecting the rate of T_4 to T_3 conversion by homogenates of liver ($p<0.01$).

Figure 11. The effect of administration of either thyroxine, estradiol benzoate, or a combination of the two, for 35 days on the rate of 5'-monodeiodination of T_4 to T_3 by hepatic (A) and renal (B) homogenates.

* Significantly different from control ($p<0.01$).

** Significantly different from all other groups ($p<0.01$).

One standard error is set off at each mean.


A
HEPATIC T_3 GENERATION (% MEAN CONTROL)
300
200
100
**
*
▭ CONTROL
▨ THYROXINE-TREATED (50μg/kg b.w./day)
■ ESTRADIOL BENZOATE-TREATED (57μg/kg b.w./day)
▧ THYROXINE + ESTRADIOL BENZOATE-TREATED
B
RENAL T_3 GENERATION (% MEAN CONTROL)
200
100
*
*
*

The liver and kidneys are the tissues with the highest T_4 to T_3 converting activity (42). However, expression of the T_4 5'-deiodinase activity of these tissues per mg of protein does not take into account a change in the amount of tissue which is available to deiodinate T_4 to T_3 *in vivo* and hence contribute to the circulating pool of T_3. Therefore, in an attempt to account for the different ratios of organ to body weight resulting from the various treatments, an index of "total" T_4 to T_3 conversion by both the liver and kidneys was calculated for each animal by multiplying the amount of T_3 generated in 30 min in a 1 ml incubation volume by the ratio of the organ to body weight of that animal. The results of this calculation were expressed for each group as a percentage of the mean control value (Table 12). The "total" rate of conversion of T_4 to T_3 by homogenates of liver from control rats was 258.6 ± 12.3 ng T_3/ml incubation volume/30 min/liver/100 g body weight. When the results were expressed in this manner, the differences in the rate of T_4 to T_3 conversion by hepatic homogenates were maintained between the groups, although the larger ratios of liver to body weight of the treated groups and the elimination of the protein concentration of the homogenates from the calculations resulted in generally larger "total" hepatic T_4 5'-deiodinase activities in the treated groups as compared to control.

The "total" rate of conversion of T_4 to T_3 by homogenates of kidney from control rats was 61.3 ± 4.3 ng T_3/ml incubation volume/30 min/2 kidneys/100 g b.w. In contrast to the expression of T_4 5'-deiodinase activity as a function of protein concentration of the homogenates, the "total" rate of T_3 generation by renal homogenates from rats treated with EB was 74% of, and not significantly different from, the

Table 12. The effects of administration of either thyroxine (T_4), estradiol benzoate (EB), or a combination of the two, for 35 days on "total" T_4 to T_3 converting activity. Values are means ± SEM.

Treatment	"Total" Hepatic T_4 5'-deiodinase activity[a]	"Total" Renal T_4 5'-deiodinase activity[a]
Control	100 ± 5	100 ± 7
Thyroxine (50 µg/kg/day, s.c.)	207 ± 9*	169 ± 6*
Estradiol Benzoate (57 µg/kg/day)	94 ± 5	74 ± 5
EB + T_4	416 ± 20**	195 ± 19+

(a) Results are expressed as percentage of mean control value. There are six values per group.

* Significantly different from control ($p<0.01$).

** Significantly different from control, thyroxine-treated and estradiol benzoate-treated groups ($p<0.01$).

+ Significantly different from control and estradiol benzoate-treated groups ($p<0.01$).

control group. The larger ratio of the kidney to body weight of the T_4 + EB-treated group as compared to the other groups resulted in a "total" rate of renal T_3 generation by this group which was significantly different from control and EB-treated groups ($p<0.01$), and not significantly different from the group treated with T_4 alone.

Discussion

The results of this study suggest that the reduced responsiveness of estrogen-treated rats to β-adrenergic stimulation is not an indirect consequence of reduced thyroid function. Food intake, body weight and the rate of hepatic T_3 generation, all of which are affected by the thyroid state of the animal, were unaffected by prolonged administration of estrogen. If EB-treated animals were physiologically hypothyroid, one might expect a reduction in these parameters. However, in agreement with previous studies the concentration of T_4 in the serum was reduced by this treatment. Indeed, administration of estradiol (0.6 to 10 μg/day) to ovariectomized rats has been shown to decrease the concentration of T_4 and increase that of T_3 in the serum in a dose-dependent manner as compared to either ovariectomized or intact female rats (136, 137). The mechanism by which administration of estrogen exerts these effects remains uncertain. It does not appear to be due to an alteration in the binding of thyroid hormones by plasma proteins, as the percentage of free T_3 and T_4 (136) or resin uptake of ^{131}I-labeled T_4 (138) is unchanged by chronic administration of E8 (2 to 25 μg/day). The possibility that the rate of secretion of T_4 by the thyroid gland is inhibited by treatment with EB cannot be answered directly by these results, although previous studies have demonstrated that thyroidal uptake (138, 139) and release (140) of ^{131}I are actually stimulated by administration of estradiol. Indeed, the enlargement of the thyroid gland accompanying the administration of EB, demonstrated in this and previous studies (139, 140), a finding not observed when T_4 was administered simultaneously (Table 10), suggests that the thyroid glands of the EB-treated rats were hyperactive, most likely responding to an increased serum concentration of TSH.

The reduction in concentration of T_4 in the serum of rats chronically treated with EB in the present study cannot be accounted for by an increase in the rate of conversion of T_4 to T_3 by the liver and kidneys, the tissues most active in this respect (42), as has been previously suggested (137). The latter study demonstrated that the increased T_3 and decreased T_4 levels in the serum of EB-treated rats was associated with an increase in hepatic T_4 5'-deiodinase activity. However, the T_4 5'-deiodinase activity of hepatic homogenates was unaffected by administration of estradiol (12 μg/day) to intact female rats for 35 days, while that of renal homogenates was actually inhibited by 44% under these conditions. The lack of change in the concentration of T_3 in the serum with administration of EB is consistent with these findings. However, the influence of estradiol on this process is complex since administration of estradiol (0.3 to 1 μg/day for 26 days) was reported to enhance the rate of conversion of T_4 to T_3 by homogenates of liver from rats ovariectomized at 20 days of age (137). However, the same dose of estradiol did not affect the T_4 5'-monodeiodinase activity in hepatic homogenates from rats ovariectomized at 40 days of age and actually decreased the activity in hepatic homogenates from male rats castrated at either 20 or 40 days of age. This is an area deserving of further study. Therefore, it remains to be elucidated whether prolonged administration of EB increases the metabolism of T_4 by another pathway, and whether hepatic conjugation and fecal loss of T_4 are increased by this treatment, as suggested previously (138, 140).

The significant interaction between administration of T_4 and EB in affecting the concentration of T_4 in the serum and the activity of the hepatic T_4 to T_3 converting enzyme is intriguing. In a previous study, when both EB and T_4 were administered together to rats which had been both ovariectomized and thyroidectomized, the concentration of T_4 was less and that of T_3 was greater than that in serum of rats receiving T_4 alone (136). In the present study, a significant increase in the concentration of T_3 above that in the serum of rats receiving T_4 alone was not observed. It is possible that the longer duration of treatment used in this study increased either the rate of deiodination or hepatic conjugation of T_3. At present, there is very little known about the factors controlling the rate of T_3 degradation. However, it seems likely that the considerably enhanced rate of conversion of T_4 to T_3 by hepatic homogenates is at least partially responsible for the reduced concentration of T_4 found in the serum of these animals. In addition, the administration of T_4 in combination with EB reduced the concentration of estradiol in the serum below that of rats receiving EB alone, although the doses of EB administered from the Silastic tubes were similar in the two groups. This finding may be due at least partially to an increase in the rate of 2- and 4-hydroxylation of estradiol in the animals receiving both EB and T_4 and is currently under study.

The reason for a differential effect of EB and EB + T_4 treatments on T_4 to T_3 converting activity of renal as compared to hepatic homogenates is not known. Although the T_4 5'-deiodinases of the liver and kidney are considered to be the same enzyme (49), the subcellular localization of the enzyme in the two tissues appears to be different. It is generally believed that the majority of the T_4 5'-deiodinase activity is located in the microsomal fraction of the liver and in the plasma membranes of the kidney (45-48). It is possible that administration of EB alone or in combination with T_4 has a differential effect at these sites. Measurement of the activity of marker enzymes specifically located either in the endoplasmic reticulum or the plasma membrane will be required to test this possibility. It should also be pointed out that omission of the protein concentration of the homogenates and inclusion of the tissue mass in the calculation of the T_4 to T_3 converting activity reduced the difference in response between the hepatic and renal enzymes. Indeed, under these circumstances the "total" rate of conversion of T_4 to T_3 by renal homogenates from EB-treated rats did not differ from that of control. Similarly, when the activity of renal homogenates from rats administered both EB and T_4 together was expressed in this manner, the ability of the kidneys of this group to contribute to the circulating pool of T_3 is increased from 129 to 195% of control. Perhaps, growth of the kidneys, induced by administration of EB, resulted in a dilution of the enzymatic activity.

Therefore, the results of this study suggest that administration of a high dose of estrogen for 5 weeks did not affect profoundly the parameters of thyroid function studied here. However, administration of a combination of EB and T_4 potentiated the effect of T_4 on hepatic T_4 to T_3 converting activity, which may be responsible for the significant reduction in concentration of T_4 observed in the serum of these animals.

Summary

The effects of administration of thyroxine (T_4), estradiol benzoate (EB), or a combination of the two, for 35 days on the concentrations of T_4, triiodothyronine (T_3) and estradiol in the serum and on the rate of conversion of T_4 to T_3 by homogenates of liver and kidney were evaluated. Administration of T_4 (50 µg/kg b.w./day) resulted in an increase and administration of EB (57 µg/kg b.w./day) resulted in a decrease in the serum concentration of T_4 as compared to control level. When T_4 and EB were administered together, the concentration of T_4 was significantly less than that in the sera of either the T_4-treated or control groups, while the concentration of T_3 was significantly greater than that measured in the serum of the EB-treated group. The concentration of estradiol in the serum was elevated considerably by administration of EB, while administration of EB and T_4 together resulted in a serum concentration of estradiol which was significantly less than that of the EB-treated group.

Administration of T_4 resulted in a rate of hepatic generation of T_3 which was 180% of control, while the rate of renal T_3 generation was 152% of control. The T_4 5'-monodeiodinase activity of hepatic homogenates was not affected significantly by administration of EB, while that of renal homogenates was inhibited by 44%. Administration of T_4 in combination with EB resulted in a rate of conversion of T_4 to T_3 by hepatic homogenates, which was 334% of control and significantly different from all other groups. The rate of renal generation of T_3 by this group of rats was 129% of control, but this was significantly less than that achieved by administration of T_4 alone.

The results of this section differ somewhat from those of the previous sections in that the T_4 5'-deiodinase activities of the liver and kidney were affected differentially under the conditions of this experiment. Also, although it has not been demonstrated consistently by the results of the previous sections, the observed alteration in the rate of conversion of T_4 to T_3 by homogenates of liver and kidney from the EB + T_4-treated group appeared to be reflected by changes in the concentration of T_4 and T_3 in the serum.

SECTION V

EFFECT OF RENAL ENCAPSULATION ON THE RATE OF 5'-MONODEIODINATION OF THYROXINE TO TRIIODOTHYRONINE IN THE RAT

Introduction

The development of experimentally-induced hypertension is associated with a reduction in thyroid function (141, 142). Studies from this laboratory have demonstrated that rats made hypertensive by renal encapsulation with latex envelopes have larger thyroid glands, a normal concentration of T_4 and a reduced concentration of T_3 in the serum as compared to normotensive controls (143). Since a portion of the daily production of T_3 in the rat results from peripheral 5'-monodeiodination of T_4 (28), it seemed worthwhile to investigate whether a reduction in the T_4 to T_3 converting activity of hepatic and renal homogenates might contribute to the reduced serum T_3 concentration of renal-encapsulated rats. In addition, to ascertain whether a deficit in this process resulted as a consequence of the development of hypertension or was a result of the renal encapsulation per se, the T_4 5'-deiodinase activity was also measured in homogenates of liver and kidneys from renal-encapsulated rats that had been treated with the angiotensin converting enzyme inhibitor, captopril, to prevent development of hypertension.

Methods

Twenty-four female rats of the Blue-Spruce Farms (Sprague-Dawley) strain weighing 220-260 g were used. They were housed in groups of 3 in a thermoregulated room maintained at 25 ± 1°C and illuminated from 7 A.M. to 7 P.M. All rats were provided with finely powdered Purina Laboratory Chow and tap water ad libitum.

The rats were divided randomly into four equal groups. Body weight and systolic blood pressure were measured, the latter by means of a Narco-Bio Systems polygraph (144). Systolic blood pressures were not different among the groups. On the following day, bilateral renal encapsulation with latex envelopes was performed on two of the groups, while they were anesthetized with sodium pentobarbital (50 mg/kg b.w., i.p.). Immediately after renal encapsulation, one of the operated groups received captopril, mixed thoroughly into the diet at a concentration of 1 g/kg of food. Captopril was kindly provided by Dr. Zola P. Horovitz, Squibb Institute, Princeton, NJ. In addition, one of the two unoperated groups served as a normotensive control, while the other received captopril (1 g/kg of food) in the diet. Daily intake of captopril under similar conditions has been shown previously to be approximately 50 mg/kg b.w./day (145).

After 5 weeks of treatment, systolic blood pressure was again measured. On the following day, the rats were sacrificed and the concentrations of T_3 and T_4 in the sera and the rate of conversion of T_4 to T_3 by homogenates of liver and kidney were determined as described in the General Methods. In addition, the hearts were removed, cleaned of extraneous tissue and weighed on a torsion balance. In this study the homogenates of liver and kidney were frozen at -65°C immediately after preparation for a maximum of 10 days prior to assay. Incubations were performed for 30 min and the results were expressed as a percentage of the mean control value. Statistical analysis of the data was performed by a two-way analysis of variance for a factorially-designed experiment (121). Comparison between individual groups was made by a t test using the pooled variance from the analysis of variance (85). Significance was set at the 95% confidence limit.

Results

Renal encapsulation for 35 days resulted in a systolic blood pressure which was significantly higher than that of control or captopril-treated groups ($p<0.01$) (Table 13). Administration of captopril to renal-encapsulated rats prevented an increase in systolic blood pressure. The body weights, ratios of liver and kidneys to body weight, and the protein concentrations of hepatic and renal homogenates were unaffected by the various treatments (Table 14). However, renal encapsulation resulted in a ratio of heart to body weight (300 ± 9 mg/100 g b.w.), which was significantly greater than that of either the control or captopril-treated groups (253 ± 12 and 238 ± 5 mg/100 g b.w., respectively) ($p<0.01$). Dietary administration of captopril to renal-encapsulated rats resulted in a ratio of heart to body weight (270 ± 7 mg/100 g b.w.), which was significantly less than that of the untreated, renal-encapsulated group ($p<0.05$) but significantly greater than that of the normotensive group receiving captopril ($p<0.05$).

Encapsulation of the kidneys, whether treated with captopril or not, resulted in a significant reduction in the concentration of T_3 in the serum ($p<0.01$), while that of T_4 was not different among the groups (Table 13). In addition, the effects of the various treatments on the rate of conversion of T_4 to T_3 are shown in Figure 12. The rates of conversion of T_4 to T_3 by hepatic and renal homogenates were 6.40 ± 0.85 and 15.82 ± 1.60 ng T_3/mg protein/30 min, respectively. The T_4

Table 13. Effect of renal encapsulation (ENCAP), treatment with captopril, or a combination of the two, for 35 days on serum concentrations of thyroxine (T_4) and triiodothyronine (T_3). Values are means ± SEM; 6 animals per group.

Treatment	Serum T_4 Concentration (µg/dl)	Serum T_3 Concentration (ng/dl)	Mean Systolic Blood Pressure (mm Hg)
Control	2.7 ± 0.3	118 ± 7	105 ± 3
ENCAP	2.7 ± 0.3	86 ± 8*	127 ± 2**
Captopril (1 g/kg food)	2.8 ± 0.3	114 ± 10	93 ± 4
ENCAP + Captopril (1 g/kg food)	2.9 ± 0.1	86 ± 4*	105 ± 6

* Significantly different from control ($p<0.05$).

** Significantly different from all other groups ($p<0.01$).

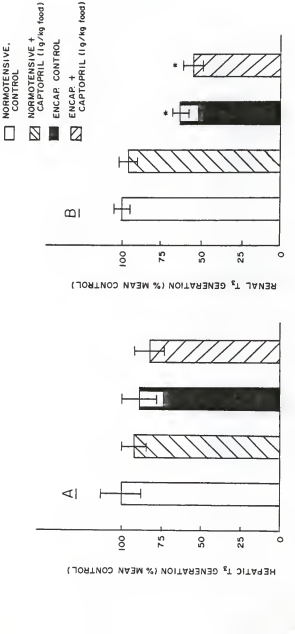
Table 14. Effect of renal encapsulation (ENCAP), treatment with captopril, or a combination of the two, for 35 days on body weights, the ratios of liver and kidneys to body weight, and the protein concentrations of homogenates of liver and kidney. Values are means ± SEM; 6 animals per group.

Treatment	B.W. (g)	Liver Wt (g/ 100 g b.w.)	Kidney Wt (mg/ 100 g b.w.)	Protein conc. of hepatic homogenate (mg/ml)	Protein conc of renal homogenate (mg/ml)
Control	274 ± 11	2.88 ± 0.17	711 ± 12	58 ± 3	26 ± 2
ENCAP	287 ± 5	3.29 ± 0.13	738 ± 27	61 ± 4	23 ± 1
Captopril (1 g/kg food)	281 ± 6	3.17 ± 0.08	686 ± 12	52 ± 2	25 ± 1
ENCAP + Captopril (1 g/kg food)	283 ± 6	3.12 ± 0.11	712 ± 58	55 ± 2	22 ± 2

Figure 12. Effect of renal encapsulation (ENCAP), administration of captopril, or a combination of the two, on the rate of conversion of T_4 to T_3 by hepatic (A) and renal (B) homogenates. Rate of conversion of each group is expressed as a percentage of the mean control value. All treatments were for 35 days.

* Significantly different from control ($p<0.01$).

One standard error is set off from each mean.


NORMOTENSIVE, CONTROL
NORMOTENSIVE + CAPTOPRIL (1g/kg food)
ENCAP. CONTROL
ENCAP. + CAPTOPRIL (1g/kg food)
A
B
HEPATIC T_3 GENERATION (% MEAN CONTROL)
RENAL T_3 GENERATION (% MEAN CONTROL)
100
75
50
25
0
*
*

5'-deiodinase activity of hepatic homogenates from the renal-encapsulated rats and those renal-encapsulated rats which were administered captopril was 86 and 79% of, but not significantly different from, control. However, renal encapsulation, with or without treatment with captopril, reduced the rate of conversion of T_4 to T_3 by renal homogenates to 63 and 55% of control ($p<0.01$). Administration of captopril did not affect significantly the T_4 5'-deiodinase activity of either hepatic or renal homogenates.

Discussion

Studies from this laboratory have demonstrated that renal-encapsulated rats are less able to concentrate their urine following a 24 hr dehydration (143) and have a reduction in the responsiveness of plasma renin activity to acute administration of the β-adrenergic agonist, isoproterenol (unpublished observation). These results suggest that the renal function of the rat is reduced by encapsulation of their kidneys. In addition, the concentration of T_3 in serum is reduced, while that of T_4 is unchanged (143). Patients with renal failure exhibit similar alterations in the concentrations of thyroid hormones in the serum, a finding ascribed to a reduction in the peripheral metabolism of T_4 to T_3 (146). Another important action of the kidney is to deiodinate T_4 to T_3 (42). Therefore, it is possible that a reduction in renal function resulting from encapsulation of the kidneys might also affect their T_4 to T_3 converting ability. Indeed, the results of this study indicate that renal encapsulation per se, regardless of whether hypertension develops, reduces significantly the rate of deiodination of T_4 to T_3 by renal homogenates and slightly reduces that by hepatic homogenates.

Since the peripheral metabolism of T_4 to T_3 accounts for a portion of the daily production of T_3 in the rat (28), it seems likely that the reduction in this process contributes to the reduced concentration of T_3 in the serum of the renal-encapsulated rat. That the T_4 5'-deiodinase of the kidney, as compared to that of the liver, is affected preferentially by renal encapsulation is most probably due to the direct effect of encapsulation on renal function. As the serum concentration of T_3 falls and hypothyroidism ensues, it might be predicted that hepatic T_4 5'-deiodinase activity, which is dependent on the thyroid state of the animal, would also be decreased significantly. This speculation awaits further study. In addition, it should be pointed out that thyroid depressing factor, a substance which originates from the kidney and has been found in high concentration in the serum of renal-encapsulated rats (147), might be inhibiting T_3 synthesis at the level of the thyroid gland and hence be responsible, at least in part, for the decrease in concentration of T_3 in the serum. Furthermore, the metabolism and hepatic conjugation of T_3 might be altered by renal encapsulation.

Studies from this laboratory have demonstrated that rats made hypertensive by renal encapsulation have a reduced responsiveness to β-adrenergic stimulation. For example, the responsiveness of tail skin temperature of rats with renal hypertension was significantly less than that of normotensive controls (57). In addition, the concentration of β-adrenergic receptors in myocardial tissue is reduced in rats made hypertensive by the removal of the right kidney and placement of a clip on the left renal artery (148). As both the responsiveness of rats to β-adrenergic stimulation and also the concentration of β-adrenergic receptors in the rat heart are dependent on the thyroid state of the animal (59, 149), it is possible that the reduction in these β-adrenergic parameters in renal hypertensive rats is a result of hypothyroidism.

Therefore, the results of this study suggest the reduced concentration of T_3 in the serum of renal-encapsulated rats may result, at least in part, from a significant reduction in the rate of deiodination of T_4 to T_3 by renal homogenates and a slight reduction in that of hepatic homogenates.

Summary

The effects of renal encapsulation with latex envelopes, administration of the angiotensin converting enzyme inhibitor, captopril (1 g/kg food), or a combination of the two, for 35 days on the rate of deiodination of thyroxine (T_4) to triiodothyronine (T_3) were evaluated in 2,000 x g supernatants of hepatic and renal homogenates. Only the renal-encapsulated group exhibited a significant increase in systolic blood pressure during the experiment. Renal encapsulation, whether or not accompanied by treatment with captopril, reduced significantly the rate of conversion of T_4 to T_3 by renal homogenates to 63 and 55% of control. The hepatic T_4 5'-deiodinase activities of these groups were slightly reduced by these treatments. Administration of captopril to normotensive rats did not affect the rate of conversion of T_4 to T_3 by either hepatic or renal homogenates. Encapsulation of the kidneys, whether or not treated with captopril, resulted in a significant reduction in the concentration of T_3 in the serum, while that of T_4 was different among the groups.

The results of this section suggest that a change in the rate of conversion of T_4 to T_3 by renal homogenates from renal-encapsulated rats may be reflected in a change in the concentration of T_3 in the serum. However, as other parameters of renal function are altered by encapsulation of the kidneys, it should be pointed out that factors, such as renal uptake of T_4 and local concentrations of glutathione, might also be affected and alter the *in vivo* deiodination of T_4 to T_3.

GENERAL DISCUSSION

A number of studies have indicated that thyroid state can affect the ability of the animal to respond to β-adrenergic stimulation. For example, the increases in heart rate, water intake, tail skin temperature and blood glucose concentration following acute administration of the β-adrenergic agonist, isoproterenol (ISO), are reduced in hypothyroid rats (59, 150). In addition, contractile responses of the rat heart are decreased in hypothyroidism (151), whereas hearts from hyperthyroid rats are more sensitive to β-adrenergic stimulation (152). Furthermore, epinephrine-stimulated production of glycerol was greater by epididymal adipocytes from hyperthyroid rats and less by adipocytes from hypothyroid rats than achieved by controls (153). The ultimate mechanisms mediating changes in β-adrenergic responsiveness under various thyroid conditions are uncertain. However, a thyroid state-dependent alteration in the concentration of β-adrenergic receptors has been observed in myocardial tissue (149) and fat cell membranes from the rat (154). Also, thyroid hormones have been demonstrated to inhibit the activity of low K_m phosphodiesterases of hepatocytes (155) and adipocytes (153). It is possible that one or both of these _in vitro_ findings may be responsible, at least partially, for the permissive action of thyroid hormones on biological responses to catecholamines.

However, the results of the present studies suggest that the changes in β-adrenergic responsiveness of rats under the various physiological and pharmacological influences described above cannot necessarily be accounted for by an alteration in the thyroid function of the animal. For example, while acclimation to cold was accompanied by an increase in the rate of 5'-monodeiodination of T_4 and in the concentration of T_3 in the serum, prolonged administration of ISO, which had been shown to mimic some of the physiological changes accompanying cold acclimation, had no effect on thyroid state. Therefore, it is possible that the increase in β-adrenergic responsiveness observed in cold-acclimated rats occurs at least partially as a result of an increase in thyroid state. However, it is also possible that an enhanced sympathetic drive increases the sensitivity of the cold-acclimated rat to β-adrenergic stimulation. Indeed, it is probable that a similar sensitization is the cause of the increased sensitivity of ISO-treated rats to β-adrenergic stimulation (84). However, the sensitization to β-adrenergic stimulation induced by chronic administration of ISO is a complex event dependent on the dose and frequency of administration and also on the β-adrenergic parameter measured. For example, Tse et al. (156) have demonstrated that the contractile responses to ISO stimulation of right ventricular strips are reduced in rats administered ISO (6 mg/kg b.w./day) for 10 days. However, administration of ISO (300 μg/kg b.w./day) for 20 days resulted in a greater increase in the rate of oxygen consumption and heart rate during an infusion of ISO than occurred in control rats (84). Also, administration of a much lower daily dose of ISO (25 μg/kg b.w.) to rats for 10 days resulted in a greater increase in heart rate, but a lower increase in water intake, than occurred in control rats following administration of ISO (157). The mechanisms by which β-adrenergic responsiveness is altered following daily administration of ISO are not yet well understood and are deserving of further study.

On the other hand, the relationship between thyroid state and β-adrenergic responsiveness in rats, which have been administered the antithyroid agent, aminotriazole (ATZ), seems relatively clear-cut. Studies performed in this laboratory have demonstrated that the reduced ability of ATZ-treated rats to increase their heart rates, water intakes and tail skin temperatures following acute administration of ISO is returned to normal by concomittant administration of T_4 (59, 150). Therefore, the reduced ability of ATZ-treated rats to respond to β-adrenergic stimulation appears to be a direct result of ATZ-induced hypothyroidism. The results reported in Section II have succeeded in defining further the extent of the depression of thyroid function elicited by administration of ATZ, and also the ability of T_4 to return the thyroid state to normal or above normal levels.

The relationship between β-adrenergic responsiveness and thyroid state in animals chronically administered theophylline is an interesting one. In vitro studies have indicated that theophylline can potentiate both the inotropic effect of norepinephrine on electrically-driven atria of the rabbit (158) and also the action of the β_2-adrenergic agonist, salbutamol, in relaxing the airway smooth muscle of the guinea pig (159). As millimolar concentrations of theophylline were used in these experiments and the synergistic effect of theophylline and the catecholamines was accompanied by a considerable elevation in cAMP content in the tissues, it seems likely that theophylline was acting by inhibition of phosphodiesterase. In addition, recent in vivo studies from this laboratory have demonstrated that the increases in heart rate and tail skin temperature following acute administration of ISO are greater in rats which have been chronically administered theophylline in the diet at a concentration similar to that used in Section III (unpublished observations). At this time it is not possible to ascertain whether the mechanisms by which theophylline increases β-adrenergic responsiveness in vivo are similar to those which increase responsiveness in vitro. However, it seems most unlikely that an increase in thyroid state is responsible for the increased β-adrenergic responsiveness of rats chronically administered theophylline since the results of the present study indicate that thyroid state is actually reduced under these conditions.

The possibility that a reduction in thyroid function in estrogen-treated rats, or in rats made hypertensive by encapsulation of the kidneys, can account for their inability to respond to β-adrenergic stimulation has been discussed previously in Sections IV and V. The results of Section IV suggest that the reduced β-adrenergic responsiveness of estrogen-treated rats occurs as a result of an influence other than hypothyroidism. At present, the mechanism of action of estrogen in this respect is unknown, although a modulation by estrogen of β-adrenergic receptor density, as observed previously in the rat cerebral cortex and myometrium (160, 161), may play a role. However, the likelihood of a relationship between thyroid state and β-adrenergic responsiveness in renal-encapsulated rats is strengthened by the finding that both of these physiological parameters are reduced in these animals. However, it should be emphasized that the observation that thyroid state and β-adrenergic responsiveness may increase or decrease concomitantly in animals in different physiological or pharmacological states does not necessarily indicate that a change in one is necessarily the cause of a change in the other. Therefore, it might be pertinent to assess β-adrenergic responsiveness in cold-acclimated or ISO-treated rats administered an antithyroid agent, or in renal-encapsulated or estrogen-treated rats administered T_4, in order to gain insight as to whether a change in the thyroid state of animals under these conditions is responsible for the observed change in β-adrenergic responsiveness.

The results of these studies have elucidated some factors which do, and indeed some which do not, influence the rate of in vitro metabolism of T_4 to T_3. It has been demonstrated conclusively that hyperthyroidism increases and hypothyroidism decreases both hepatic and renal T_4 5'-deiodinase activities. Acclimation to cold increased the rate of this process most likely as a result of the increased thyroid state of animals under these conditions. Administration of aminotriazole and theophylline decreased the rate of conversion of T_4 to T_3 by hepatic and renal homogenates most probably indirectly by a reduction in thyroid hormone secretion, although the possibility of a direct action of the latter compound on the enzyme should not be ruled out. The β-adrenergic system does not appear to play a role in regulation of the rate of conversion of T_4 to T_3. This finding aids the interpretation of the other data, in that the possibility that a change in β-adrenergic responsiveness (as has been described previously to occur in the various physiological and pharmacological states used in these experiments) might actually bring about an alteration in the rate of peripheral 5'-deiodination of T_4 can be discounted. Administration of a combination of estrogen and T_4 resulted in a dramatic increase in hepatic T_4 5'-deiodinase activity, although administration of estrogen alone was without effect. On the other hand, renal encapsulation appeared to preferentially decrease renal T_4 5'deiodinase activity, at least at the time after encapsulation studied here.

However, the results of these studies have indicated that caution should be taken in relating changes in the rate of in vitro conversion of T_4 to T_3 to that which occurs in vivo, especially when attempting to account for changes in the serum concentrations of T_3 and T_4. As mentioned in Section I, the in vitro model measures enzymatic activity under conditions in which the concentrations of substrate and cofactor are sufficient to maintain the activity of the T_4 5'-monodeiodinase at an optimal level. The limiting factor for the generation of T_3 under these conditions is the concentration of the enzyme. Therefore, it seems most likely that changes in the activity of the T_4 5'-deiodinase, as observed under the various conditions in these studies, reflect a change in the concentration of the enzyme, although the possibility that there is a treatment-induced alteration in the responsiveness of the enzyme to the stimulatory cofactor cannot be ruled out.

However, the limiting factors for the deiodination of T_4 to T_3 in vivo are not known with certainty, and in vitro studies can give only limited information in this respect. For example, as the activity of the T_4 5'-deiodinase of hepatic microsomes from fasted rats is reduced below control levels, it has been suggested that a decrease in the concentration of the enzyme is at least partially responsible for the lower concentration of T_3 found in the sera of these animals (94). In another case, the low concentration of nonprotein sulfhydryl groups in homogenates of liver from ovine fetuses and the ability of the addition of dithiothreitol to raise the T_4 5'-deiodinase activity of fetal lambs to adult levels have suggested that the concentration of the endogenous cofactor, glutathione, may be the limiting factor for peripheral production of T_3 (50). However, the two studies cited above, and indeed the present studies as well, lack both the control systems associated with intact cellular and organ structure and the ability to respond to metabolic and hormonal perturbations.

Therefore, in a very recent study Jennings et al. (95) have investigated those factors which control the conversion of T_4 to T_3 in the isolated perfused rat liver, a preparation in which cellular and structural integrity is maintained. The perfused liver readily extracted T_4 from the perfusion medium and converted it to T_3, indicating the presence of T_4 5'-deiodinase activity. It was demonstrated that the production of T_3 was a function of those factors which also increased the tissue uptake of T_4. These included an increase in the size of the liver, an increase in the perfusion flow rate, an increase in the concentration of T_4 or a decrease in the concentration of albumin in the perfusate. The finding that the production of T_3 increased progressively with increasing concentrations of T_4 (from 3 to 60 μg/dl) in the perfusate suggests that the enzymatic capacity to deiodinate T_4 to T_3 exceeds the quantities of endogenous T_4 (from 3 to 6 μg/dl) available for deiodination in vivo. Therefore, it was concluded that the uptake of T_4 by the liver, rather than the concentration of the T_4 5'-deiodinase enzyme in the liver, might be the limiting factor for regulation of T_4 to T_3 deiodination by the rat liver under these conditions.

If the conclusions of the latter study can be extrapolated to relate to in vivo conditions, then a change in the concentration of the T_4 5'-deiodinase enzyme would be expected to result in much less of an effect on T_3 generation in vivo than has been considered previously. Therefore, homogenate preparations, although allowing study of the enzymatic and biochemical aspects of deiodination, might be limited in their ability to evaluate the possibility that T_3 generation might be regulated by the availability of substrate, as well as by the prevailing enzyme activity. Therefore, in the future it might be useful to utilize an isolated perfused organ system to evaluate whether a change in the uptake of T_4 by the liver or kidneys might be a factor contributing to the changes in the production of T_3 resulting from any of the various physiological and pharmacological states described in the present studies. It would also be most interesting to utilize such a system to compare the deiodinating capacities of the liver and kidneys in terms of their contribution to the overall production of T_3. Analysis of this question using traditional techniques has been hindered by the need to account for differences in the concentration and subcellular location of the T_4 5'-deiodinase enzymes, differences in the protein concentrations of homogenates, and in the size of and blood flow to these tissues, and possibly capillary transit time and concentration of glutathione as well. As it has been suggested that the T_4 5'-deiodinase of the kidney is located in the plasma membrane, it is possible that the uptake of T_4 is less critical in limiting T_4 to T_3 conversion by this tissue. Indeed, if the T_4 5'-deiodinase is located at the cell surface of the kidney, it may represent a physiological mechanism to allow efficient production of circulating T_3 from T_4 without the obligatory penetration by T_4 of the cell membrane and intracellular space.

The results of these studies have also demonstrated the difficulty in correlating the rate of deiodination of T_4 to T_3, as measured _in vitro_, to the concentrations of T_4 and T_3 observed in the serum. The relatively small, and in some cases negligable, changes in concentration of T_3 and T_4 in the serum of rats, in which the _in vitro_ T_4 to T_3

converting activities of their tissues showed considerable variation, is puzzling and the problem cannot be clarified by the available literature. The possibility that the uptake of T_4 rather than the amount of enzyme may be the limiting factor for deiodination of T_4 to T_3 in vivo would suggest that in vitro deiodination and serum hormone concentrations would correlate much better under the conditions where the uptake of T_4 and amount of T_4 5'-deiodinase might increase or decrease concomitantly. Additionally, as pointed out in each section, thyroidal secretion and hepatic conjugation of thyroid hormones may well be altered under the various conditions. Furthermore, the factors which affect the rate of deiodination of thyroid hormones by alternate pathways, especially deiodination at the 5-position (of T_4 to rT_3 and of T_3 to 3,3'-diiodothyronine) are unknown and are the subjects of intense study at the present time. As any one or more of these factors might have been altered by the conditions of these studies, it is perhaps not surprising that a correlation between in vitro deiodination of T_4 to T_3 and the concentrations of these hormones in the serum was not consistently seen.

Finally, a question arises as to what contribution local monodeiodination of T_4 to T_3 or plasma T_3 play in occupation of the nuclear T_3 receptors of individual tissues. Chopra (42) demonstrated that seven of eight different tissue homogenates exhibited T_4 to T_3 converting activity (none being detected in homogenates of lung). Therefore, fivefold greater free concentration of T_4 than T_3 in the plasma (39) combined with high intracellular deiodination of T_4 to T_3 might contribute the major proportion of the biological activity of the thyroid hormones. Indeed, this appears to be the case in the anterior pituitary, in which over half of specifically bound nuclear T_3 originates from

from intrapituitary conversion of T_4 to T_3 (162). However, local monodeiodination of T_4 to T_3 appears to play a minor role in regulating thyroid hormone-dependent activities in the liver and kidney. This process provided only 28 and 14% of the nuclear T_3 in these tissues, respectively, the remainder being derived from plasma T_3. Therefore, the finding of Kaplan and Utiger (81) that thyroid-dependent parameters of the liver and kidney can be considerably reduced in thyroidectomized rats, whose serum concentrations of T_4 are undetectable but whose levels of T_3 are not different from control, cannot be explained by a reduction in local T_4 to T_3 conversion and reduced availability of intracellular T_3, but must result from another unknown mechanism. Possibly a decrease in the rate of uptake of T_3 by these tissues or a decrease in the concentration of nuclear receptors for T_3 might contribute.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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August 1981

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